JOURNAL OF AGRICULTURAL RESEARCH

VOL. 69 WASHINGTON, D. C., AUGUST 1, 1944 No. 3

ANATOMICAL AND CYTOLOGICAL STUDIES ON BEET MOSAIC[1]

By KATHERINE ESAU

Assistant botanist, California Agricultural Experiment Station

INTRODUCTION

The anatomy of the sugar beet (*Beta vulgaris* L.) affected with mosaic has been inpestigated by several workers, but some results are contradictory. Robbins (*46*)[2] described necrosis of the vascular tissues and of the adjacent parenchyma in mosaic beets, and Roland (*47*) saw gummosis in the phloem tissue of beets affected by "jaunisse," which he regarded as similar to beet mosaic. Böning (*7*), Schaffnit and Weber (*48*), and Sirotina (*54*), on the other hand, reported no phloem necrosis in mosaic beets. Schaffnit and Weber (*48*) found foreign bodies in the phloem of mosaic sugar beets, but Sirotina (*54*) noted such bodies in healthy plants also. According to the modern concept, a mosaic virus is not limited to the phloem and produces few or no abnormalities in this tissue (*5*, *21*); it therefore appeared pertinent to reexamine the phloem of beets affected with mosaic. The first part of the present paper compares the phloem of healthy and mosaic-diseased sugar beets.

The second part deals with the structure of the mesophyll in mosaic beet leaves, particularly with plastid abnormalities. According to many workers (*7–15*, *25*, *32*, *34*, *42*, *53*, *57*), the yellow areas in leaves affected with mosaics are hypoplastic and contain small chloroplasts reduced in numbers as compared with healthy mesophyll or with the dark-green areas of mosaic leaves. Some workers strongly emphasize that underdevelopment is the only abnormality of the yellow areas, that cell contents are not destroyed, and that the affected cells continue to develop until they become more nearly like those of the green areas (*7*, *8*, *10*, *11*, *12*, *13*). Although recognizing hypoplasia as the principal disease symptom in the yellow areas, other workers record also some injury to the contents, particularly the plastids (*9*, *14*, *25*, *32*, *44*). The present study is concerned especially with the question whether underdevelopment alone explains the peculiarities of the yellow areas in mosaic leaves, or whether destructive changes also occur in them.

[1] Received for publication March 25, 1943.

[2] Italic numbers in parentheses refer to Literature Cited, p. 114.

MATERIALS AND METHODS

The mosaic-diseased sugar-beet material was obtained from artificially inoculated greenhouse plants grown at Riverside, Calif.,[3] and from naturally inoculated plants in fields of the Sacramento Valley. Healthy leaves were collected from greenhouse plants and in fields that showed no mosaic in the years when this disease was not prevalent. Symptom-free plants in severely affected fields were also sampled. The material was examined partly in the fresh state in freehand sections mounted in water, partly after treatment by a common paraffin method. Tap and distilled water were tried for the fresh sections, but the former was selected as the less injurious medium. Several killing solutions were employed in the paraffin method, mainly formalin-aceto-alcohol mixtures, Randolph's modified Navashin fluid (*35, p. 45*), and a bichromate-chromic-osmic acid solution according to Champy (*40, p. 37*). The paraffin sections were cut 7 to 10 microns thick and were stained with Heidenhain's haematoxylin and safranine as used previously by the writer (*22*)[4]. This staining method gives a good wall differentiation and also shows cytoplasmic details. The stain may be variously balanced so as to have either the haematoxylin or the safranine predominating in the cytoplasmic structures. In detail, the schedule is as follows:

(1) Place slides, with paraffin removed, in distilled water.
(2) Change distilled water 5 times during 10 minutes.
(3) Mordant in 4-percent aqueous ferric ammonium sulphate for 10 minutes.
(4) Transfer to distilled water, and change the latter 4 or 5 times during 10 to 20 minutes.
(5) Transfer into a weak solution of haematoxylin prepared by putting 10 drops of stock solution (2.5 gm. of haematoxylin crystals in 100 cc. of 95-percent ethyl alcohol) into a Coplin staining dish with tap water or with distilled water made slightly alklaline with sodium bicarbonate. Stain until the walls appear bluish. The time varies with the kind of material, the age of the organ, and the fixing solution used. From 10 to 20 minutes was required in the present investigation.
(6) Rinse in tap water.
(7) Stain 6 to 24 hours in a dilute safranine solution prepared by adding 5 to 6 drops of stock solution (1 gm. safranine O in 100 cc. of 50-percent alcohol) to a Coplin dish with tap water.
(8) Destain in 50-percent alcohol, and carry through higher alcohols to xylene. Treat each slide individually.
(9) Mount in balsam.

The larger photographs were taken on Isopan film, the smaller on Finopan film. Wratten filters B–58 (green) and 44 (minus red) were used in photography. The drawings were prepared with the aid of a camera lucida. All photographs were made from paraffin material, the drawings mostly from freehand sections.

EXTERNAL SYMPTOMS OF BEET MOSAIC

As several workers have indicated (*7, 43, 46, 54, 58*), the yellow-green mottling of the mosaic beet leaves shows various patterns and different degrees of paleness and distinctness of the yellow areas. Masking of symptoms also occurs in this disease. Figure 1 shows a

[3] This material was supplied by Dr. C. W. Bennett, Division of Sugar Plant Investigations, U. S. Department of Agriculture.
[4] This method of staining was originally recommended by Dr. R. H. Wetmore, of Harvard University.

healthy leaf and a mottled mosaic leaf of greenhouse plants from Riverside, and figure 2 illustrates the common patterns of mottling found on mosaic beets in a field near Davis. Leaf *A* in figure 2 has the diffuse type of mottling characteristic of older leaves. In leaf *B* the yellow areas are comparatively large and sharply delimited. This pattern somewhat resembles that of the mosaic leaf in figure 1. The

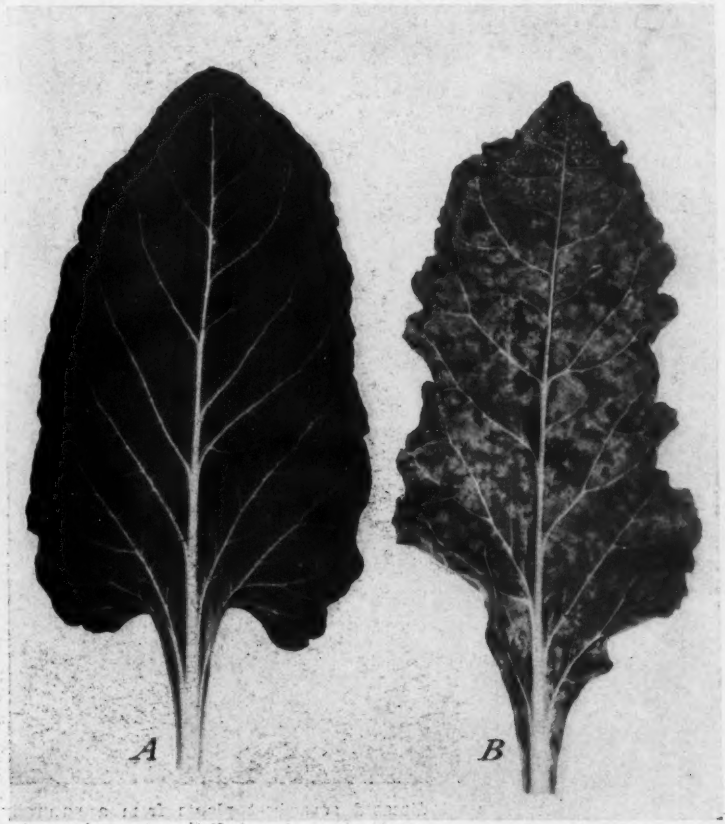


FIGURE 1.—*A*, Healthy leaf and *B*, mosaic-diseased leaf from sugar-beet plants grown in a greenhouse at Riverside, Calif. The diseased plant was artificially inoculated with the mosaic virus. (Photograph furnished by Dr. C. W. Bennett.) ×1.

contrast between the yellow and green areas is particularly sharp in leaf *C* (fig. 2). In leaf *D* the yellow areas have the shape of small roundish spots.

ANATOMY OF THE PHLOEM OF MOSAIC LEAVES

The present writer (*18*, *19*) has already described the development and structure of the phloem in healthy sugar-beet leaves. Briefly,

the functioning phloem as seen in a transverse section of a bundle (pl. 1, *A*) is a small-celled tissue composed of sieve tubes, very small companion cells, and phloem parenchyma cells. These last are the largest in the tissue. The older part of the primary phloem which ceased to function constitutes the bundle cap (pl. 1, *A*, left). Within

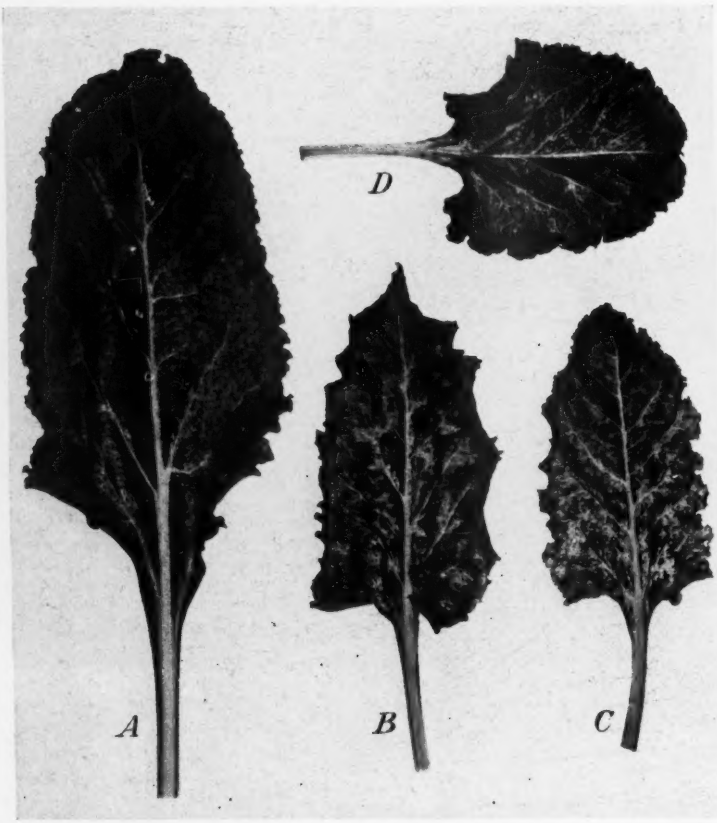
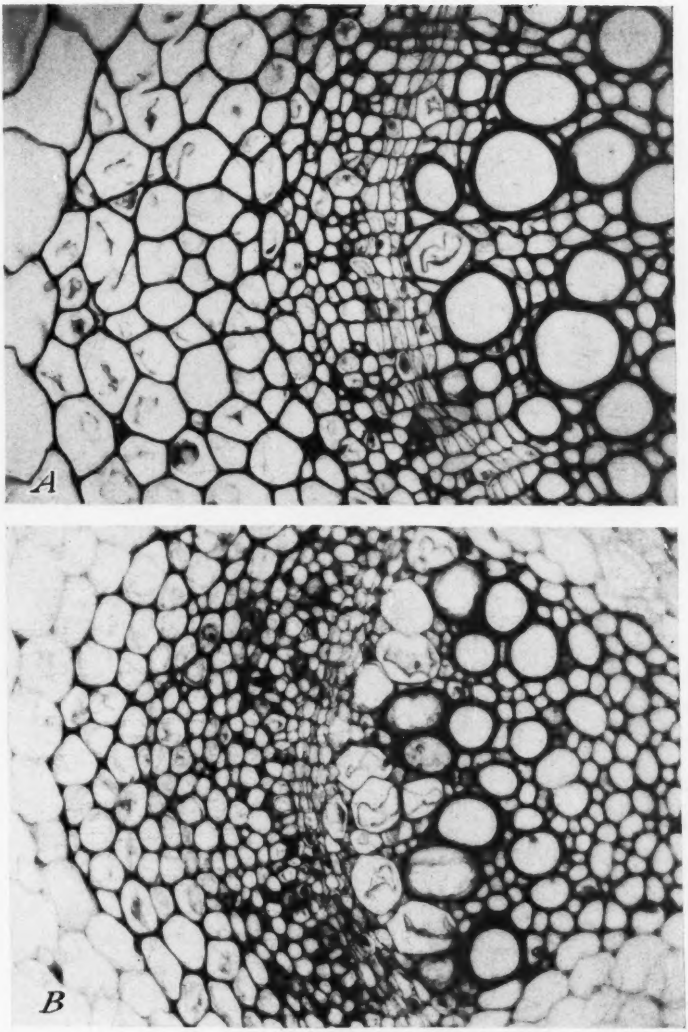


FIGURE 2.—Leaves from mosaic-diseased sugar-beet plants from a naturally infected commercial planting near Davis, Calif.: *A*, Diffuse type of mottling characteristic of older leaves; *B*, yellow areas comparatively large and sharply delimited; *C*, contrast between yellow and green areas particularly sharp; *D*, yellow areas appearing as small roundish spots. × ½.

it the remnants of crushed sieve tubes and companion cells seem like deeply stained wall thickenings. The intact cells of the cap are the much-elongated phloem cells, which develop rather thick cellulose walls after the conducting elements of the phloem are obliterated.

Younger and older leaves from many severely affected plants grown in greenhouse and outdoors were examined for phloem structure. No abnormalities were observed in the vascular tissues, when the leaves



Transverse sections of vascular bundles from healthy (*A*) and mosaic-diseased (*B*) beet leaves. × 290.

showed no necrosis externally. A vascular bundle of a young, severely mottled leaf from a field-grown plant appears in plate 1, *B*. This bundle is younger than that in plate 1, *A*. It shows normal phloem and an immature bundle cap in which the different stages in obliteration of the sieve tubes and companion cells are conspicuous. The phloem of sugar-beet leaves affected with curly top strikingly contrasts with that of the mosaic leaves. Curly top induces hypertrophy and hyperplasia in the phloem of affected plants. The tissue resulting from these growth abnormalities sharply deviates from the normal phloem (*18*, *20*). Eventually it collapses, and a gumlike substance accumulates among the dead cells.

Certain mosaic leaves used in the present study showed necrosis of various tissues. Thus necrosis of phloem, xylem, mesophyll, and epidermis was observed in the local lesions that sometimes appeared at the place of inoculation and in yellow areas along the veins through which the virus passed from the place of inoculation before systemic infection occurred. The necrosis was a collapse of cells that appeared to have developed normally, accompanied by an accumulation of deeply staining material. Judging from these instances of phloem necrosis, this abnormality may occur under certain conditions in mosaic beet, but is not a constant or specific symptom and does not indicate a close relation of the mosaic virus to the phloem. In contrast, phloem degeneration in curly top diseased plants is a constant primary symptom and is one indication of the close association between the virus and the phloem of the host. The other pathologic changes in mosaic and curly top beets point in the same direction. The stunting of plants and the malformations on leaves in curly top appear to be secondary symptoms that can be explained as resulting from disturbances in the phloem tissue (*18*, *20*). The mesophyll abnormalities in mosaic beets, on the other hand, can be well interpreted as primary symptoms induced by the virus in this tissue.

It is difficult to explain the necrosis of entire mesophyll cells and even of the vascular tissues observed in the mosaic material just described while such necrosis is absent in leaves that develop after the systemic infection has become established. Conceivably, at the place of inoculation and along the veins where the virus is first moving out of the places of inoculation, the virus concentration is particularly high and produces an initial shock, causing some necrosis. Further studies on this problem would be of interest.

Robbins (*46*), who considered malformations and necrosis of tips of leaves in mosaic sugar beets to be symptoms of mosaic, sectioned such leaves and found in them necrosis of phloem and other tissues. From these observations he concluded that beet mosaic induced phloem necrosis. The malformations and tip necrosis that he described are not, however, symptoms of beet mosaic and frequently occur in plants not affected by this disease.[5]

While the condition of the phloem in mosaic beets was being considered, this tissue and others were examined also for the bodies described by Schaffnit and Weber (*48*) and Sirotina (*54*) in mosaic and healthy beets. These "elytrosoma" are said to be commonly spindle-shaped or very much elongated, often of the same size as the nuclei or

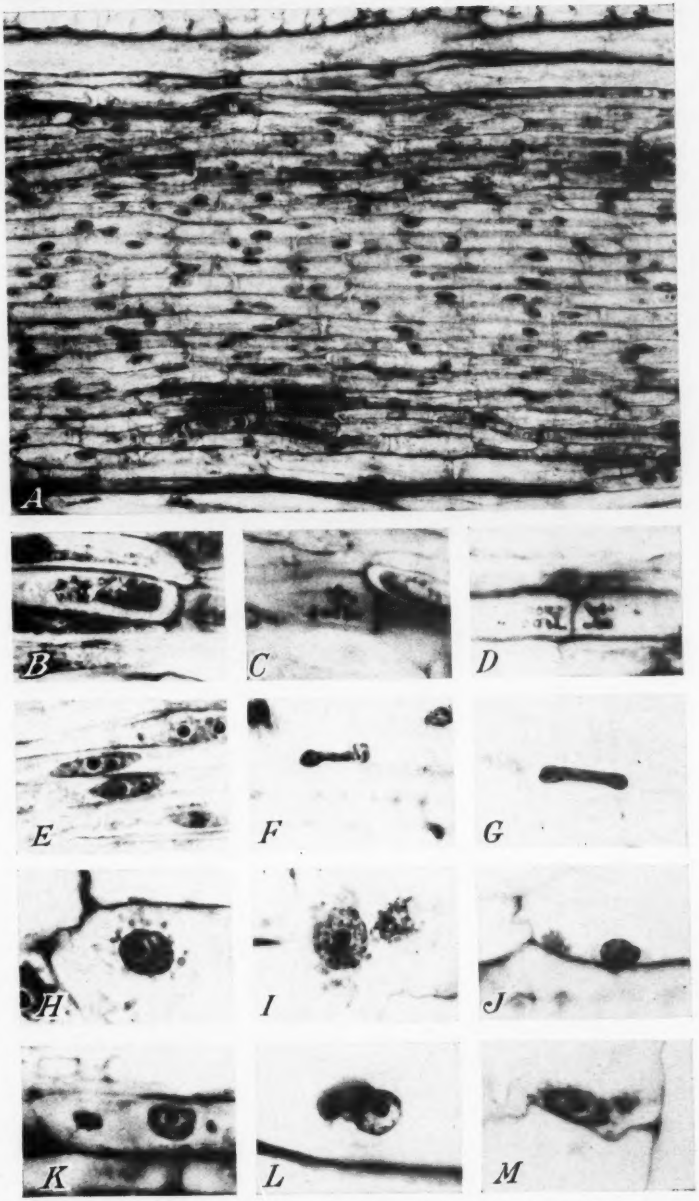
[5] Personal communication by Dr. C. W. Bennett and Dr. Eubanks Carsner, Division of Sugar Plant Investigations, United States Department of Agriculture.

larger. They either are homogeneous or they contain spheroidal granules. Schaffnit and Weber emphasized that these bodies occurred in undifferentiated phloem of young leaves. In the present study no bodies of the sort were observed. Plate 2, *A*, illustrates a tangential longitudinal section of procambium and young phloem of a bundle from a young sugar-beet leaf that showed severe mottling. There are no foreign bodies in evidence. The somewhat elongated nuclei are the only conspicuous structures within the procambial cells in the center of the field. In some cells two nuclei appear to be present, but only as the result of an optical effect. The procambial cells are radially compressed, and in tangential sections the nuclei of two cells may occur at very close levels. The procambial walls are not thick enough to obstruct the light materially. The phloem appears above and below the procambium in plate 2, *A*. This tissue has certain characteristic protoplasmic elements sometimes erroneously related to diseases (*19*, *21*). Plate 2, *B*, shows slimy accumulations of an immature sieve-tube element; plate 2, *C* and *D*, the sieve-tube plastids of mature sieve-tube elements. The "elytrosoma," as described in the literature (*48*, *54*), do not resemble the slime bodies or the sieve-tube plastids. They may be compared with some nuclei in the elongated cells of the vascular bundles, such as those in plate 2, *E* to *G*, but are not supposed to show the same internal structure as the nuclei.

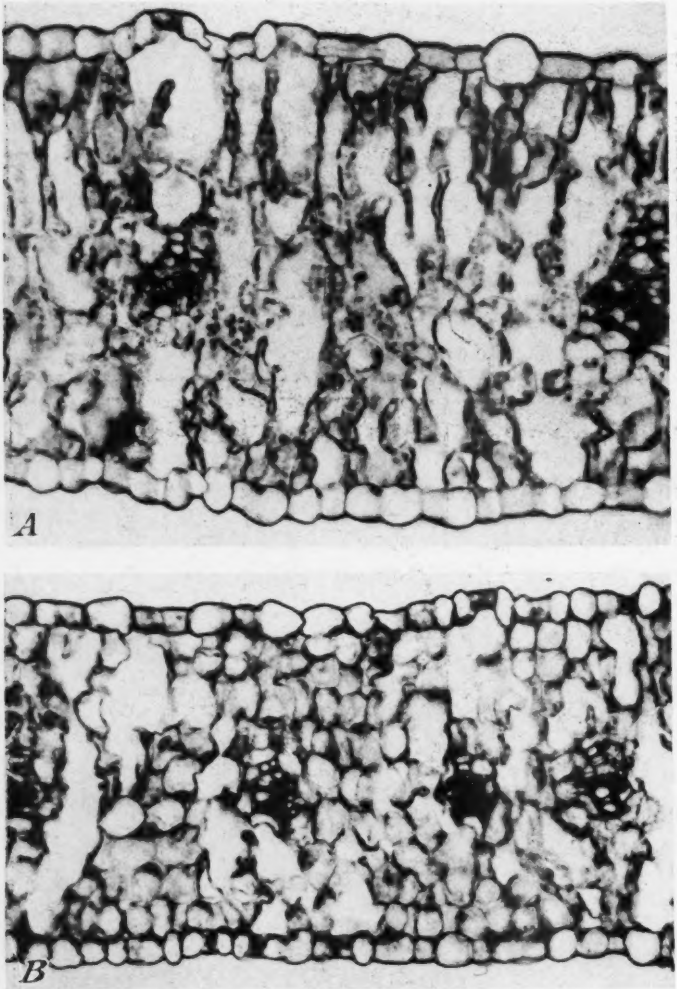
EFFECT OF MOSAIC UPON THE MESOPHYLL

According to Böning (*7*), the beet mosaic resembles other mosaics in that it induces mottling by retarding the differentiation of isolated spots in the mesophyll. These spots become the yellow areas as the leaf develops. The yellow areas are thinner, with fewer intercellular spaces and less chlorophyll, than the green areas. Böning (*7*), who attributed all these abnormalities to undervelopment of the mesophyll and chloroplasts, recorded no destructive changes in mosaic leaves.

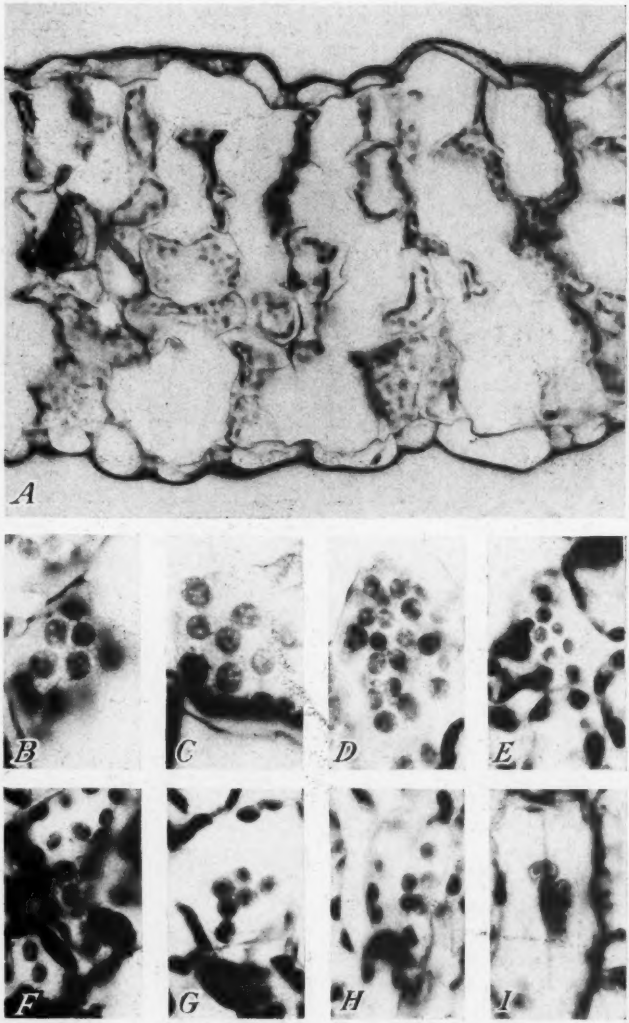
The material used in the present study confirms the view that the mesophyll in the yellow areas is hypoplastic. Plate 3 illustrates a section from a green area (*A*) and a yellow area (*B*) of the same medium-sized mosaic leaf. In each photograph the palisade region appears above, the spongy below. The yellow area, as contrasted with the green, shows certain juvenile characteristics: the mesophyll is not differentiated into palisade and spongy layers, its cells are more or less isodiametric, and the intercellular system is rather poorly developed. Its chloroplasts, furthermore, are indistinct—a feature considered in detail elsewhere in this paper. A dark-green area (pl. 3, *A*) may appear thicker than healthy mesophyll from leaves of comparable age or older (pl. 4, *A*); that is, the green areas may be hyperplastic. The degree of difference between them and healthy mesophyll varies considerably, however; they may be no thicker than the healthy leaf. The differences between the yellow and the green areas also vary. In a very young leaf the difference in the rate of development of the green and yellow areas will not have had time to produce variations in leaf thickness. In old leaves, on the other hand, the two areas may have the same thickness because the effect of the disease is insufficiently severe. According to Böning (*7*), the yellow areas continue to develop so that eventually they become more nearly



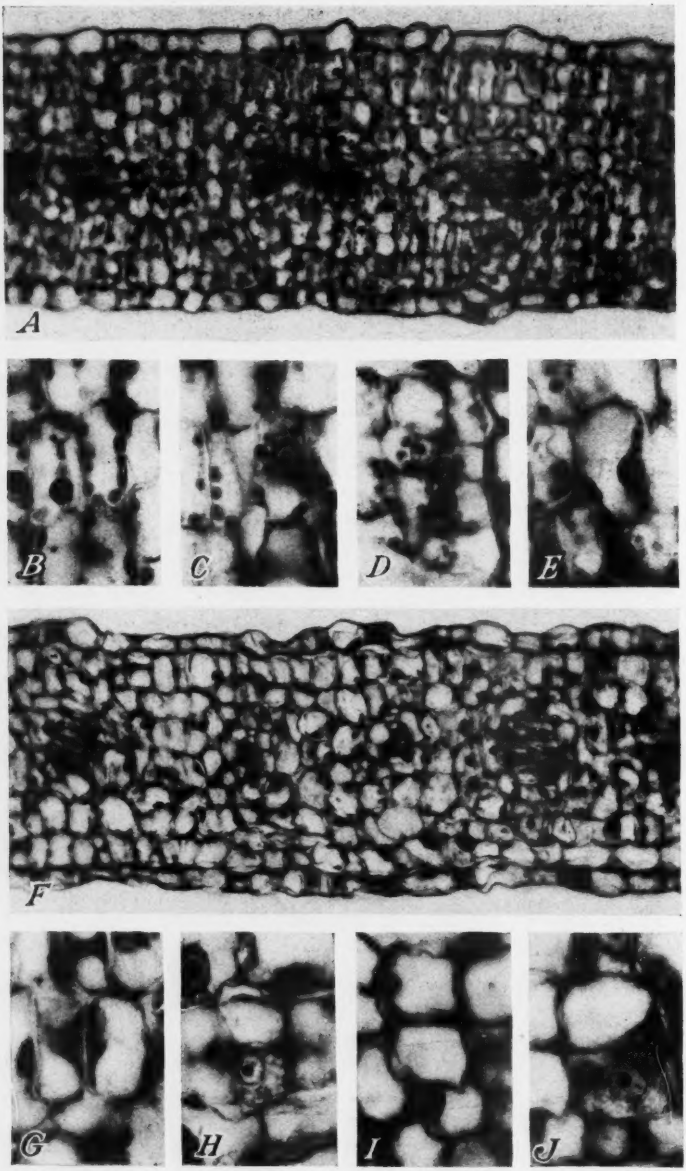
A, Tangential longitudinal section through procambium of a vascular bundle of a young mosaic-diseased beet leaf. × 290. *B*, Sieve-tube slime; *C* and *D*, sieve-tube plastids; and *E*, procambial nuclei from section as in *A*. *F* and *G*, Nuclei in xylem parenchyma of mosaic beet leaves. *H* to *M*, Plastids in different stages of coalescence and nuclei from various cells in mosaic beet leaves. *B* to *M*, × 900.



Transverse sections of a green (*A*) and yellow (*B*) area of a medium-sized beet leaf severely affected by mosaic. ×290.



A, Transverse section of mesophyll of healthy medium-sized beet leaf. ×290. *B* to *H*, Mesophyll chloroplasts, and *I*, epidermal plastids and nucleus from healthy (*B* to *F*) and symptom-free (*G* to *I*) beet leaves. *B* to *F*, fixed according to Navashin; *G* to *I*, according to Champy. *B* to *I*, ×900.



A, Transverse section of a green area; and *B* to *E*, several cells from such areas of very young mosaic beet leaves. *A*, ×290; *B* to *E*, ×900. *F*, Transverse section of a yellow area; and *G* to *J*, several cells from such areas of the same leaves as in *A* to *E*. *F*, ×290; *G* to *J*, ×900.

like the green. Some old leaves with mild mottling used in the present study showed only slight histologic differences between the yellow and green areas, but these leaves might have been infected while they were partly differentiated and in consequence might have been little affected by the virus. Whether a leaf showing as striking a difference between the light and dark areas as in plate 3 would largely eliminate this difference during further development still remains to be determined. Often the yellow areas show such profound cytologic disorganization that the possibility of recovery seems doubtful. Developmental studies with repeated samplings of the same leaves are desirable in this connection.

In leaves developing after the systemic infection has occurred the future yellow areas are characterized by suppression of cell divisions, whereas the cells of the future green areas continue to divide actively or perhaps even divide at abnormally high rates if the resulting green area becomes hyperplastic at maturity. In the early stages of development, consequently, the green area (pl. 5, *A*) has smaller but more numerous cells and appears less differentiated than the yellow area of the same leaf (pl. 5, *F*). Later, when the green area assumes its more or less mature characteristics, the yellow area appears as an underdeveloped portion of the leaf (pl. 3).

EFFECT OF MOSAIC UPON THE PLASTIDS

PLASTIDS OF HEALTHY AND SYMPTOM-FREE LEAVES

Plastids easily react to various stimuli by changes in their appearance, and the modifications within the range of normal variability are not clearly separated from those indicating a pathologic state. Because the problem of changes in the physical state of plastids is so complex (*29*, *52*, *61*), the plastids of the healthy and symptom-free beets were studied in detail before diseased material was considered.

When plastids were examined in fresh sections, two criteria were used in identifying living cells: the occurrence of protoplasmic streaming and the presence of mitochondria. Though the absence of streaming does not necessarily indicate that the cell is dead, its presence is generally regarded as a sign of life. Mitochondria, on the other hand, are very fragile; their presence indicates that the cell has not been materially injured in preparation. These bodies were not identified by applying specific tests; rather, they were located and interpreted as mitochondria by comparing published descriptions of mitochondria with bodies observed in cells of a variety of plants, including the beet. Furthermore, different fixatives were applied under the microscope. Some were acetic acid fixatives, known to cause a dissolution of mitochondria; others were bichromate fixatives, said to preserve them. The mitochondria as identified by these means in the beet commonly appear as dumbbell-shaped rods with swellings on both ends. Frequently the elongated mitochondria separate into pairs of spherical bodies. With time this separation becomes more common, so that after 2–3 hours of observation the spherical bodies predominate. The mitochondria are carried by the streaming cytoplasm and often show also an oscillatory movement. Other common bodies comparable in size with mitochondria occur in the living cells. These, being much more highly refractive than the mitochondria, are probably oil globules.

The appearance of the nucleus may also be used as a criterion of cell condition. In a living cell, with ordinary illumination, the nucleus

FIGURE 3.—Plastids and nuclei in freehand sections from mosaic-free beet plants. *A*, Chloroplasts of a mesophyll cell. × 1,360. *B*, Chloroplasts crushed by pressure on cover glass. × 1,360. *C*, Leucoplasts and nucleus from pith of hypocotyl. × 1,400. *D*, Chloroplasts and nucleus from petiole. × 1,400. *E*, Chloroplasts and nucleus from pith of seedstalk. × 1,400. *F*, Same as in *E* after treatment with a bichromate fixative. × 1,400

shows no structure; but in a dead cell it has a granular reticulum or is granular throughout.

According to recent literature, chloroplasts may appear either granular or homogeneous, both conditions being normal (*4, 31, 60, 62, 63*). The chloroplasts of the beet leaves, whether from the mesophyll (fig. 3, *A*) or from other chlorophyll-containing parenchyma (fig. 3, *D*), often show a distinctly granular structure. The granules are arranged in somewhat orderly fashion (fig. 3, *A*); they appear dark at one focus, light at another (fig. 3, *D*); and when the plastid is mechanically crushed by pressure on the cover glass, the mass remains granular (fig. 3, *B*). At other times the chloroplasts are very finely granular, with few, somewhat large granules showing no orderly arrangement (fig. 5, *A–C*). No entirely homogeneous chloroplasts were observed in this study.

The chloroplasts of the mesophyll may be more or less flattened and appressed to the walls. Sometimes they are spread out so much as to crowd each other and assume somewhat angular outlines (fig. 5, *B*). Or they may be rounded off and not at all crowded (fig. 3, *A*; 5, *C*). Different cells in the same section may show different degrees of flattening of the chloroplasts. Though these differences in shape might partly account for the apparent variations in plastid size, actual differences in plastid mass probably occur also. The photomicrographs in plate 4, *B* to *H*, illustrate variations in size of chloroplasts upon fixation and dehydration in sections of mature leaves. The plastids in plate 4, *B* to *D*, were found in different cells of one leaf section; those in plate 4, *E* and *F*, in cells of another; those in plate 4, *G* and *H*, in cells of a third. Even within a given cell, plastids may vary in size (fig. 5, *A*). Apparently in relation to the time of collection, the chloroplasts either did or did not contain starch grains. The chloroplasts in plate 4, *B* to *F*, show starch grains.

The chloroplasts of the parenchyma cells surrounding the vascular bundles of the medium and large veins are smaller and paler than the chloroplasts of the mesophyll. They tend to aggregate about the nucleus (fig. 3, *D* and *E*) like the leucoplasts that occur in the colorless pith parenchyma of the root crown or flowering stalk (figs. 3, *C*, and 4, *A*). The aggregation of certain plastids around the nucleus has been recorded in the literature (*29, 52*). In living cells the leucoplasts or the pale chloroplasts appear to touch the nucleus (figs. 3, *C* to *E*). When killed, however, the nucleus shrinks, and the distance between it and the plastids is somewhat increased. Figure 3, *F*, shows the same nucleus and plastids as figue 3, *E*, but after treatment with Navashin's fixing fluid. In the fixed cell of figure 4, *A*, also, the nucleus is somewhat shrunken.

The leucoplasts characteristically undergo amoeboid changes in shape. Figure 4, *B*, shows the changing shape of one leucoplast during 15 minutes. The amoebid shape of leucoplasts is also evident in figure 3, *C*, taken from a living cell, and in figure 4, *A*, showing a cell treated with a mitochondrial bichromate fixative. Though the chloroplasts display less tendency toward amoeboid changes, they may have colorless processes that alter their shape and size (fig. 3, *E*).

The living leaf sections are eventually injured by the tap water used for mounting them. Sooner or later after being mounted, certain cells exhibit vesiculation of plastids. The plastids (and the mitochondria) swell and are converted into vesicles while the nucleus shrinks (fig. 4, *C*). When a chloroplast swells, it sometimes appears

as though split in two halves still attached to the vesicle (fig. 4, *D*); or a green granular mass dispersed over the surface of the vesicle makes the latter appear in optical section like a ring with green particles adhering to it all around. Whether these particles are within the

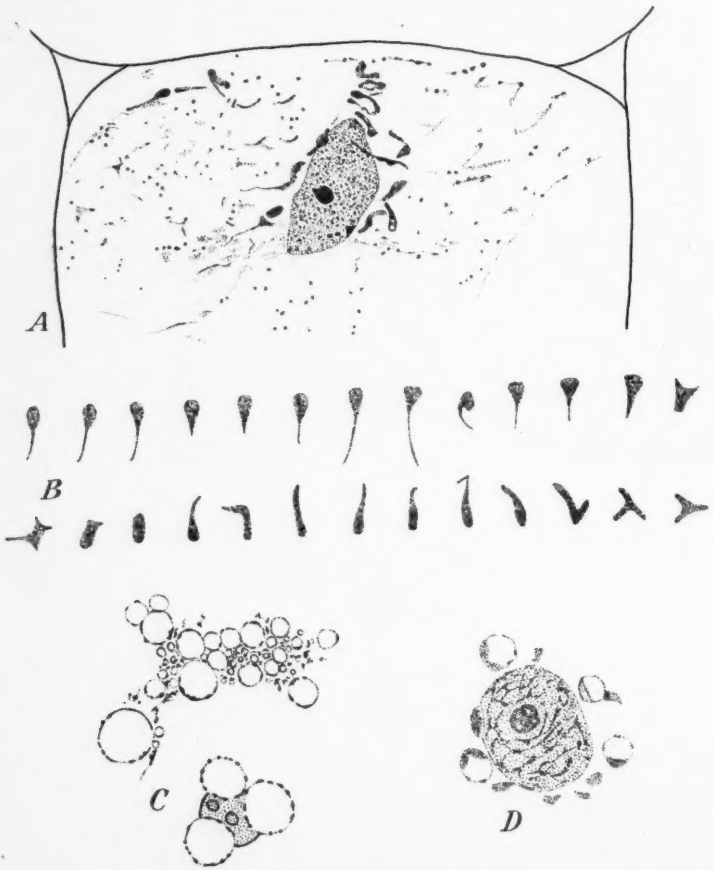
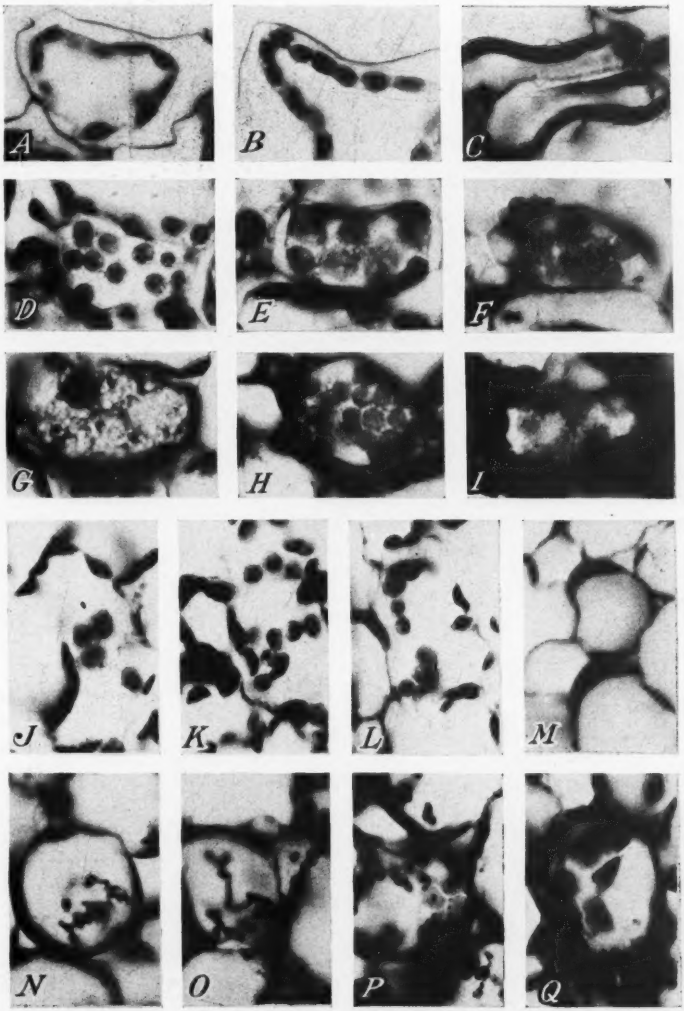


FIGURE 4.—Nuclei and plastids from mosaic-free beet (*A–C*) and *Chenopodium murale* (*D*) plants. *A*, Protoplast of parenchyma from hypocotyl treated with a bichromate fixative and embedded in paraffin. × 1,200. *B*, Series of free-hand drawings of the same living leucoplast from parenchyma cell, showing amoeboid changes that occurred during 15 minutes. About × 1,200. *C*, Shrunken nucleus and swollen chloroplasts and mitochondria affected by tap water used for mounting. × 1,200. *D*, Shrunken nucleus and swollen chloroplasts treated with a bichromate fixing solution after they were affected by tap water. ×1,200

membrane of the vesicle and form bulges on its surface or whether they adhere on the outside has not been ascertained. The literature does not clearly answer this question (*36*, *41*, *61*). One can fix the vesicles by treating the section with a fixing fluid under the microscope



A and *B*, Chloroplasts from a healthy leaf. *C*, *E* to *I*, and *M* to *Q*, Cells from yellow areas of mosaic beet leaves. *D*, and *J* to *L*, Cells from green areas of mosaic beet leaves. *A* to *I*, Fixed according to Navashin; *J* to *Q*, according to Champy. All × 900.

599130—44——2

(fig. 4, *D*); but they have not been observed in paraffin material. In fresh sections they eventually burst, leaving a greenish granular mass behind. When during cutting the chloroplasts are extruded from the cells, they vesiculate much quicker than the plastids within the cells. There is also a variation in the time when this phenomenon appears in different cells of the same section. In some cells, probably injured in cutting, vesiculation appears at once; in others not for various lengths of time; or the protoplast may disintegrate without swelling of plastids.

PLASTIDS OF MOSAIC LEAVES

The chloroplasts of the dark-green areas of mosaic leaves (fig. 5, *E* and *F*, and pl. 6, *D*, *J* to *L*) generally resemble the chloroplasts of healthy and symptom-free leaves (fig. 5, *A* to *C*; pl. 4, *B* to *H*). The two kinds of plastids show similar variations in size, degree of granulation, degree of spreading and flattening; both may or may not have starch. Plastids in sections of the green areas of mosaic leaves tend to show somewhat more pronounced vesiculation than the healthy mesophyll.

The chloroplasts of the yellow areas undergo pathologic changes of different degrees. Generally, if these chloroplasts are present as discrete bodies, they are light-colored. Starch is occasionally present if the plastids are not much modified. The latter apparently vary in size as much as the chloroplasts of the dark-green areas or of healthy leaves; but no statistical studies of plastid size were made during the present investigation. Chloroplasts in yellow areas show a much greater tendency toward vesiculation than those in healthy mesophyll or in dark-green areas of mosaic leaves. Numerous cells in water mounts of sections of yellow areas appear as though filled with foam (fig. 5, *G*), the vesicles sometimes deforming each other. More or less fragmented chloroplasts are associated with the vesicles. Eventually the latter burst, and a granular pale-green or yellowish mass remains.

Chloroplasts that do not become vesiculated tend to be spread out very thin or to coalesce. Sometimes they appear amoeboid in shape (fig. 5, *D*). The different appearance of the coalescing plastids suggests the following sequence in the process. The plastids are clumped within a cytoplasmic sheath that appears very tenuous (fig. 6, *A*). Then the chloroplasts lose their sharp outlines (fig. 6, *C*), and finally the plastids and the cytoplasm together appear as a greenish mass closely appressed to the cell wall (fig. 6, *B*). At this stage the cells still respond with plasmolysis to hypertonic sugar solutions. Eventually, in fresh sections, the coalesced material fragments. At the same time the nucleus becomes granular, and crystals appear in the cell. Some highly refractive bodies, usually one per cell, may be present before the cell becomes completely disorganized (fig. 6, *A*, above); but there may be similar bodies in symptom-free sections also. Usually the plastids appear in different stages of coalescence when the section is mounted, but sometimes fusion occurs during observation. Thus of the two cells in figure 6, *D*, the lower showed fusion of plastids about 30 minutes after the section was prepared. The plastids clumped together, became coarsely granular, and then fused (fig. 6, *E*). Cytoplastic streaming ceased, and crystals appeared near the nucleus. The slide was placed in a refrigerator overnight, and

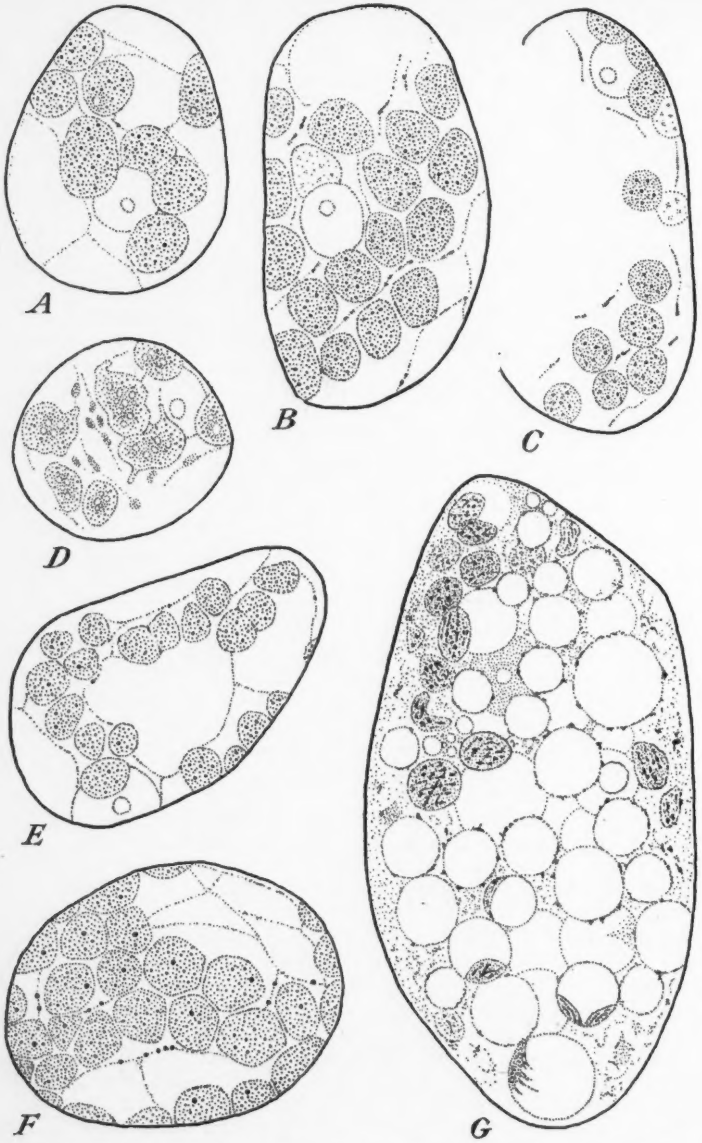


FIGURE 5.—Mesophyll cells from freehand sections of sugar-beet leaves mounted in tap water. *A* to *C*, From a plant free of symptoms; *D* and *G*, from yellow areas, and *E* and *F*, from green areas of mosaic leaves. Further explanations in text. All × 1,450.

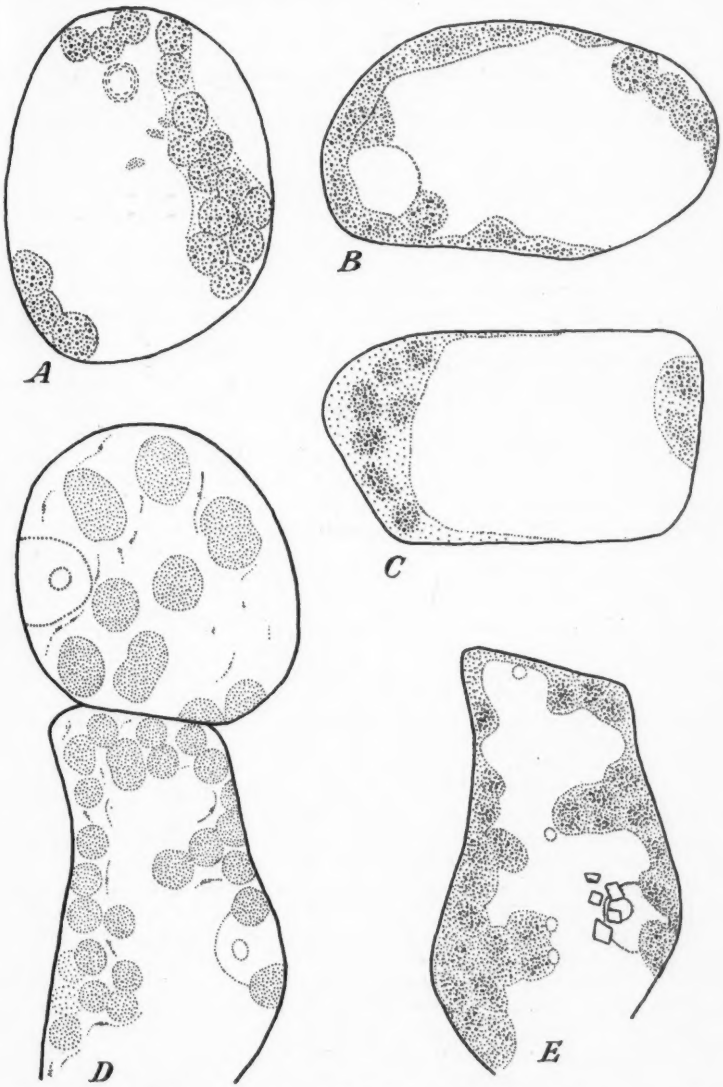


FIGURE 6.—Mesophyll cells from mosaic beet plants mounted in tap water. The chloroplasts in *A* to *C* and *E* are in different stages of coalescence. The chloroplasts in *D* are not coalesced, but the lower cell in *D* appeared as in *E* 30 minutes after being mounted. All × 1,450.

next morning the upper cell was still intact. In the lower cell, however, the protoplast was completely disintegrated. The two cells in figure 6, *D*, were originally drawn to illustrate the characteristic variation in chloroplast size in the mesophyll of mosaic and diseased beet leaves. The cells in figure 6, *D*, occurred in the border between a yellow and a green area.

Coalescence of chloroplasts in yellow areas is evident also in paraffin material. Plate 6, *C*, shows coalesced plastids within the cytoplasmic sheath lining the walls of two cells. Plate 6, *E* to *I*, gives several face views of partly and entirely fused chloroplasts. The spreading of plastid material in earlier stages of coalescence is obvious in plate 6, *E*, a view which strikingly contrasts with that of the rounded-off plastids from a dark-green area in plate 6, *D*. The occurrence of coalesced plastids in paraffin material and in parts of fresh sections somewhat removed from cells obviously injured in preparation indicates that the chloroplasts of yellow areas may be in various stages of coalescence in intact leaves.

Rather thoroughly degenerated cells such as are encountered in paraffin sctions of yellow areas appear in plate 6, *M* to *Q*. For contrast, cells from the dark-green areas of the same leaf are shown in plate 6, *J* to *L*. In plate 6, *M*, the chloroplasts, nucleus, and cytoplasm form together a protoplasmic sheath closely appressed to the wall. This sheath is rather thick where the nucleus occurs. The cell in plate 6, *N*, contains darkly staining deposits, probably tannic in nature, as well as the photoplasmic sheath lining the wall. The cell in plate 6, *O*, is similar except that it shows also a vesiclelike structure at the lower right. Another vesicle appears in the cell above. The origin of these vesicles has not been determined. They are probably not degenerated nuclei, because often more than one vesicle occurs in a cell, whereas the chloroplasts are part of the coalesced mass lining the wall. In plate 6, *P* and *Q*, the plastids appear like irregular amorphous masses smeared on the wall. The nuclei were not discernible in cells shown in plate 6, *N* to *Q*.

It seems doubtful that cells so disorganized as those in plate 6, *M* to *Q*, could recover and become more nearly like those of the dark-green areas (pl. 6, *J* to *L*). The literature indicates, furthermore (see Discussion), that fusion of plastids, sometimes called agglutination (*39*), is an irreversible process. The degenerative changes observed in the chloroplasts of the yellow areas, especially their pronounced tendency to swell and burst in water mounts or to fuse into amorphous masses, indicates a profound alteration in their physical state. The entire protoplast appears to be changed, since the cells in the yellow area usually show no shrinkage of protoplasts (pl 6, *C*, *M* to *P*) such as commonly appears in paraffin sections of healthy (pl. 6, *A* and *B*) and dark-green cells (pl. 6, *L*).

Since the partly or completely coalesced chloroplasts of diseased cells stain lightly, in photography the contrast must be artificially increased in order to bring out the plastids, as in plate 6, *P* and *Q*. The diffuse condition of the plastids and their light staining explains the difference in cytological appearance between the dark-green and the yellow areas. In the former the chloroplasts are discrete, more or less deeply stained bodies (pl. 3, *A*); in the latter they are obscure (pl. 3, *B*).

The degree of chloroplast degeneration in yellow areas varies in different leaves and also in different parts of the same leaf or even of the same yellow area. These differences might be caused by a delay in virus entry into some areas or cells as compared with others, or by variations in resistance of cells to the virus. A combination of the two factors might be assumed also. There is evidence, however, that the mosaic virus affects the chloroplasts in different stages of development. Scarcity of plastids and suppression of cell division clearly differentiate the yellow (pl. 5, *F–J*) from the green (pl. 5, *A–E*) areas in leaves that show the earliest signs of mottling. Judging from the youngest sections in plate 5, *B* and *G*, the chloroplast development might be suppressed or delayed in the yellow areas. At least, it is difficult to decide whether the appearance of cells as in plate 5, G, results from inhibition of chloroplast development or from disorganization of structures already present in the cells. According to Sheffield (*53*), plastid primordia usually are destroyed in solanaceous hosts having aucuba mosaic, whereas mature plastids are not affected by the virus. No special attempts to distinguish plastid primordia from plastids proper were made in the present study. Certainly, however, the yellow areas are deficient in plastids at the earliest stages of their differentiation (pl. 5, *G*). Slightly later (pl. 5, *H* to *J*) some plastids develop, but they stain lightly and are somewhat diffuse. In still older leaves the yellow areas may have more or less affected chloroplasts (pl. 6, *E*, *F*, *H*), or the latter may be thoroughly disorganized (pl. 6, *G*, *I*, *M*, to *Q*). Probably the more severely degenerated cells are those affected by the virus in their earliest stages of development.

The evidence that choloroplasts of partly developed leaves are affected by the mosaic virus was obtained from leaves that were inoculated or that received the virus from the inoculated leaf in advanced stages of development.[6] Yellow spots sometimes develop at the place of inoculation. In the present study such spots showed signs of injury, apparently caused by rubbing the leaves during inoculation; the epidermal cells on the upper side of the blade and sometimes also the deeper layers of mesophyll were necrotic, and wound-healing reactions had set in beneath the necrotic cells. As is well known, wound-healing involves hypertrophy and hyperplasia accompanied by loss of chloroplasts, if the latter are present in the affected tissue. Many other cells, however, in the yellow areas somewhat removed from the places of superficial necrosis also showed degeneration of chloroplasts. The latter appeared very small or diffuse or were fragmented, or the entire protoplast was replaced by flocculent material. Darkly staining bodies of cholorplast size, possibly necrotic chloroplasts, also occurred in these sections; and some entirely necrosed cells were dispersed among those that were still intact. Similar degenerative changes occurred in the yellow strips of mesophyll that were located along the larger veins through which the virus moved out from the place of inoculation; also in the yellow mesophyll associated with a lateral vein in a leaf that was in an advanced stage of development when the virus entered the plant through another leaf. All the sections mentioned above were compared with similar sections of mesophyll from symptom-free parts of the same leaves. These showed no plastid abnormalities. Plate 7, *A*, illustrates a section of yellow mesophyll

[6] This material was prepared by Dr. C. W. Bennett.

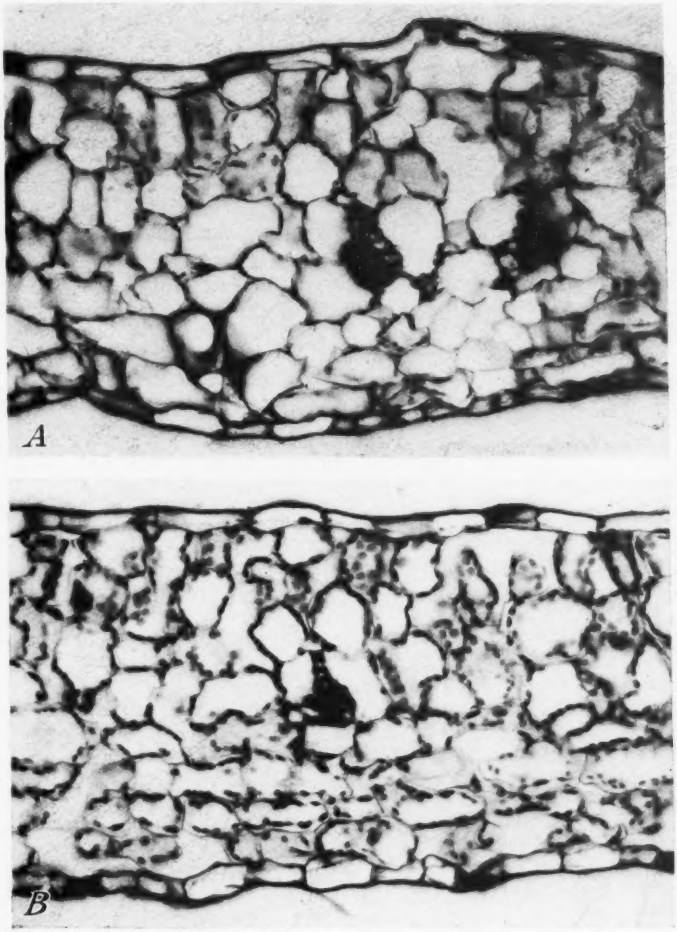
occurring along a lateral vein through which the virus passed when the leaf was partly grown. The scarcity of chloroplasts strikingly contrasts this section with the mesophyll in plate 7, *B*, taken from a similar position in the opposite, symptom-free side of the same leaf. The observations just given indicate that cells in different stages of differentiation may be affected by the virus.

Though the chloroplasts of the mesophyll show the most striking effect of the mosaic, the plastids of the parenchyma along the larger vascular bundles apparently also become disorganized. Such cells sometimes contain flocculent or reticulate bodies that lie more or less close to the nuclei (pl. 2, *I*, *J*, and *M*). These bodies may also occur in parenchyma cells of the vascular bundles (pl. 2, *K*) and in epidermal cells (pl. 2, *L*). The bodies seemingly result from coalescence of plastids. As already mentioned, plastids of some kinds of parenchyma and of the epidermis frequently aggregate near the nuclei (pls. 2, *H*, and 4, *I*). This tendency would explain the proximity of the bodies to the nuclei. Views like the one in plate 2, *I*, suggest transitional stages between the aggregation of plastids near the nucleus and their fusion into a body. The absence of plastids in cells containing the bodies is still another indication that these bodies are related to plastids. Morphologically the bodies in mosaic beet parenchyma resemble the X-bodies described in other mosaic diseases.

DISCUSSION

In general, the results of the present study of the mosaic sugar beet agree with other workers' conclusions that the yellow areas in the mottled leaves of plants affected by mosaic viruses are deficient in chlorophyll and tend to be more or less thin as compared with the dark-green areas (*7*, *8*, *9*, *14*, *15*, *25*, *32*, *42*, *55*, *65*, *66*). The mosaic beet, furthermore, in common with many other mosaic plants (*9*, *14*, *42*, *65*, *66*), shows certain juvenile characteristics in the yellow mesophyll: the latter is not differentiated into palisade and spongy parenchyma and its cells are cuboidal in shape and rather closely packed. In other words, these areas are underdeveloped, or hypoplastic, as compared with the green areas of the diseased leaves and with the mesophyll of the healthy leaves. Usually the yellow areas are described also as thinner than healthy mesophyll. An exception appears to be mosaic *Datura stramonium*, in which, according to Smith (*55*), the yellow areas are approximately as thick as the healthy mesophyll. In the mosaic beet the yellow areas, though tending to be comparatively thin, varied in thickness and were sometimes as thick as the green areas of the same leaf or as the mesophyll of healthy leaves.

The dark-green areas of mosaic leaves are often described as thicker than healthy leaves (*14*, *15*, *42*, *55*). Unusually long, narrow palisade cells are mentioned as occurring in these areas. Goldstein (*25*), on the other hand, regards the dark-green areas as hypoplastic, though less so than the yellow areas. According to Clinch (*9*), the green spots of potato leaves affected with crinkle are thicker than healthy leaves, but in potatoes affected with certain other mosaics they are of the same thickness as the healthy mesophyll. In the present study the green areas of some mosaic beet leaves were markedly



A, Yellow mesophyll occurring along a vein through which the mosaic virus passed when the leaf was partly grown. The chloroplasts have been partly destroyed in this mesophyll. × 290. *B*. Mesophyll taken from a similar area on the other side of the same leaf as in *A*, the side that showed no mosaic symptoms. A normal complement of chloroplasts appears in this section. × 290.

thicker than healthy mesophyll of similar age, whereas in other leaves the green mesophyll resembled the healthy.

According to the review just given with regard to thickness of mesophyll, the degree of difference between the yellow and the green areas on the one hand and between the healthy and diseased leaves on the other may vary considerably. Workers have also presented conflicting data on the severity of cytologic effects of mosaic viruses, such as abnormal starch accumulations (*7*, *9*, *16*, *34*, *42*, *53*, *57*, *65*, *66*), or the inhibition of plastid development, or the occurrence of chloroplast destruction (*7–15*, *25*, *32*, *34*, *42*, *44*, *53*, *57*). Such variations may be expected. Viruses naturally differ in virulence, and the severity of symptoms is affected by the age of the leaf at the time of infection. Thus Stone (*57*) found little effect of the mild mosaic virus upon the leaf thickness in potato, whereas Hoggan (*32*), using other mosaic viruses, observed the usual reduction in thickness of the yellow areas in solanaceous hosts. As Goldstein's (*25*) study, on the other hand, clearly shows, leaves infected when still very young exhibit more marked external symptoms and internal abnormalities than those that were partly differentiated when the virus entered them. Various environmental conditions also affect the expression of symptoms, as is evidenced by the masking of symptoms that occurs under certain conditions of light and temperature in many mosaics. The divergent reports on starch accumulation must be judged in the light of the observations that viruses delay both accumulation and removal of chloroplast starch. The susceptibility of symptom expression to various internal and external factors might well explain the conflicting data on the anatomic and cytologic abnormalities in mosaic diseases.

As was observed in the introduction to this paper, some workers attribute the abnormalities of the yellow areas of mosaic leaves to underdevelopment only; others describe destructive changes in the affected protoplasts as well. The present study supports the view that the cell contents are disorganized in mosaic leaves. Clinch's (*9*) conclusion that definite physiological disturbances act in conjunction with an initial underdevelopment seems to picture correctly the abnormalities in the light areas of mottled leaves.

Hypoplasia in yellow areas merits further attention from students of pathological anatomy. In partly or fully developed leaves the yellow mesophyll usually appears more juvenile than the green. Judging from certain developmental studies on mosaic plants (*12*, *14*, *34*), cell division in early stages of differentiation of mosaic leaves is suppressed in the yellow areas. Similar observations were made in the present study. Iwanowski (*34*), furthermore, pointed out that the yellow areas precociously develop intercellular spaces, so that in young leaves these parts seem to consist of a tissue older than the still-dividing, compact green mesophyll. Grainger and Heafford (*27*) found that certain groups of mesophyll cells in leaf primordia of mosaic tobacco underwent earlier vacuolation than other adjacent ones; they suggested that these cells would become the yellow areas in the mature leaf. This suggestion, if correct, would indicate that the cells in the yellow areas complete differentiation before those of the green. The early appearance of intercellular spaces and the suppression of cell divisions in these areas point in the same direction. Apparently, then, in the early stages of differentiation, the yellow areas are hyperplastic, since

certain phenomena of tissue maturation appear in them earlier than elsewhere in the mesophyll. Later they appear hypoplastic because they do not complete the typical differentiation of the mesophyll tissue.

Certain workers interpret the mottling of mosaic leaves as the result of an uneven spread of virus in the affected leaves (*12*, *25*, *53*). Light areas arise in those parts of the leaf that are still very young when invaded by the virus, whereas green areas develop if the virus is late in reaching the cells. Despite good evidence that yellow areas contain a higher virus concentration than the green leaf parts (*26*, *33*), other phenomena besides the uneven spread of the virus might cause the mottling in mosaic leaves. Cells within the same tissue, even those occurring side by side, may react differently to the same stimuli or to injurious agents. Alexandrov (*1*) found that the tendency of sunflower chloroplasts to swell under certain climatic conditions varies within the same part of the leaf. According to Beauverie (*3*), fusion and degeneration of epidermal chromoplasts in *Ranunculus* petals treated with saponin may occur in one cell and not in another adjacent to it. Finding that certain zones die off in leaves of *Bryum capillare* treated with α-rays, while others remain alive, and that there are no transitions between the two zones, Biebl (*6*) assumed that the dead parts were less resistant. Gratzy-Wardengg (*28*) observed that starvation caused the plastids to disintegrate in most cells of fern prothallia, but that certain "green islands" of cells retained their chloroplasts. These cells showed particularly high osmotic values and were able to undergo regenerative changes. According to Schwarz (*51*), *Selaginella* grown at temperatures of 10° C. and below develops variegated leaves, the plastids degenerating in the white areas. Islands of green cells may remain, however, within the white tissue, indicating that individual cells vary in their responses to low temperatures. Sometimes one of two guard cells of the same stoma may remain green while the other turns white. At the present stage of our knowledge of the virus-host relations, we cannot ignore the possibility that cells of the same age and location in the plant body vary in susceptibility to the virus.

With regard to the plastid abnormalities observed in the mosaic beet leaves, both vesiculation and fusion of plastids have been described by many workers. These abnormalities may be induced by various factors. In general, living plastids respond easily with vesiculation to changes in the osmotic equilibrium (*2*, *4*, *29*, *38*, *49*, *61*). The swollen vesiculated chloroplasts may endure for many days, reacting to hypertonic and hypotonic solutions with changes in volume (*36*, *39*). As a rule, the vesiculated plastids eventually burst, leaving behind a greenish granular mass. Knudson (*37*), however, reported having obtained a stable form of vacuolated plastids in *Polypodium aureum* gametophytes that occurred in progenies from X-rayed spores.

Several workers have recorded vesiculation of chloroplasts in fresh sections of mosaic-diseased leaves. Iwanowski (*34*), who found that vesiculation in mosaic tobacco occurred most rapidly in the yellow areas, took this fact to indicate a lower resistance of these plastids. Similarly, Hoggan (*32*) saw plastids of the yellow leaf parts in mosaic potatoes swelling into colorless vesicles. She suggested that this phenomenon might bear on the formation of spheres in tomato mosaic reported by Sorokin (*56*). The liquefaction of the chloroplasts de-

scribed by Eckerson (*17*) in tomato mosaic also somewhat resembles vesiculation. According to Beauverie (*2, 4*), vesiculation of chloroplasts in potatoes affected with leaf roll becomes more pronounced with increase in severity of the symptoms. Cook (*12*) and Sheffield (*53*), observing vesiculation of chloroplasts in healthy and virus-diseased leaves, concluded that it had no bearing on the diseases. The results of the present study—observations on the rapid vesiculation and disintegration of chloroplasts in the diseased leaves and on the relatively greater stability of the chloroplasts in the symptom-free leaves—support the concept that resistance of the plastids to changes in the osmotic conditions of the environment is reduced by the presence of the virus.

The spreading of plastids, their loss of characteristic shape, the clumping and fusions observed in the mosaic-diseased beet leaves, all indicate a physiological disturbance. Similar plastid abnormalities have been frequently described in the literature. Although constituting pathologic phenomena, they are not sharply separated from normal variations in shape and consistency of plastids. A spreading and flattening of chloroplasts, so that they crowd each other and become angular in outline, is apparently a normal state of the chloroplasts in the mesophyll, which, however, alternates with a rounding off in relation to changes in light, temperature, moisture, and other conditions (*6, 52, 64*). Amoeboid changes in shape with the production of pseudopodialike processes are known to occur in chloroplasts and particularly in leucoplasts under apparently normal conditions (*23, 29, 31, 38, 39, 49, 52, 61*). Plastids may produce pseudopodia in light and round off in darkness (*45*). Pseudopodia may be formed by plastids when the cells are exposed to X-rays or are treated with plasmolyzing agents (*6, 24, 37, 39*).

Spreading and change in the shape of plastids, however, may be the beginning of pathologic changes that result in their coalescence and final breakdown (*3, 6, 28, 39, 41*). The fusion of plastids into an apparently viscous mass, sometimes called agglutination (*39, 41*), has been induced by treatment with hypotonic and hypertonic solutions, alcohols of low percentages, other organic substances, α-rays, and neutral red (*3, 6, 39, 41, 59, 61*). Though agglutination is usually described as an irreversible pathologic change, Knudson (*37*) reported agglomerated and fused plastids as stable forms in gametophyte progenies from spores of *Polypodium aureum* treated with X-rays.

Clumping and fusion of plastids have rarely been recorded by students of mosaic diseases. Dickson (*14*) spoke of agglomerations of chloroplasts and coalescence into irregular green masses in severe mosaic infections. Nelson (*44*), describing bean mosaic, mentions flattening of the stroma of chloroplasts and their eventual collapse into a coherent mass of viscous, pale-yellow or colorless material with a highly refractive surface. Loss of contours, clumping and fusion of plastids, were also observed in noninfectious chlorosis and variegations (*30, 50, 51*).

Judging from the data on plastid abnormalities, these structures can react similarly to many different injurious agents and environmental effects. The vesiculation and coalescence of chloroplasts in mosaic leaves cannot, therefore, be regarded as responses to a specific action of the mosaic virus. They are symptoms of physiologic dis-

turbances within the diseased cells—disturbances that might be brought about by various agencies, as well as viruses, and which reduce the stability of the plastids.

SUMMARY

Sugar-beet leaves with systemic symptoms of mosaic used in the present study showed no abnormalities in the phloem; this tissue was not degenerated, nor did it contain any foreign or abnormal bodies. Apparently the mosaic virus is less intimately related to the phloem than is the curly top virus, which constantly and primarily induces phloem degeneration in the affected plants.

The mottled mesophyll of the mosaic-diseased plants shows the usual characteristics of mesophyll of mosaic leaves. The yellow areas tend to be thinner than the green areas and show certain juvenile characteristics: their cells are nearly isodiametric and rather closely packed, with no typical differentiation into palisade and spongy layers. Early in development the yellow areas show scanty cell divisions, so that at first they have larger cells and appear more mature than the small-celled green areas in which divisions are still continuing. The green areas resemble healthy mesophyll or appear hyperplastic. The degree of difference between the yellow and the green areas, on the one hand, and between the diseased and healthy leaves, on the other, varies considerably.

The chloroplasts of the yellow areas undergo pronounced pathologic changes. In young cells they are deficient in number, possibly because of delayed development or destruction of plastid primordia. In older cells the chloroplasts are pale and fragile; they readily respond with swelling and disintegration to the contact with tap water. Or the chloroplasts are diffuse and irregularly shaped, and stain lightly. In the most severely diseased cells the chloroplasts fuse into amorphous masses. Nuclei may be absent from such cells. According to the literature, similar degenerative phenomena occur in plastids exposed to many different injurious agents. Apparently they are symptoms of physiologic disturbances which are caused within the diseased cells by the presence of virus, but which are not necessarily responses to any specific action of the virus.

LITERATURE CITED

(1) ALEXANDROV, W. G.
1929. ZUSTAND UND TÄTIGKEIT DER CHLOROPLASTEN BEI VERSCHIEDENEN KLIMATISCHEN BEDINGUNGEN. Protoplasma 6: [429]–437.

(2) BEAUVERIE, J.
1928. QUELQUES ASPECTS DE LA DÉGÉNÉRESCENCE DES PLASTES, APPLICATIONS AU PARASITISME. Rev. Gén. Bot. 40: 206–225, 264–276, illus.

(3) ———
1938. ÉTUDES DE CYTOLOGIE EXPÉRIMENTALE: ACTION DE LA SAPONINE, DE LA LÉCITHINE ET DE L'OLÉATE DE SODIUM SUR LES CHROMOPLASTES DES PÉTALES DE RENONCULES. Rev. de Cytol. et de Cytophysiol. Vég. 3: [65]–79, illus.

(4) ———
1938. LA STRUCTURE GRANULAIRE DES GRAINS DE CHLOROPHYLLE. Rev. de Cytol. et de Cytophysiol. Vég. 3: [80]–109, illus.

(5) BENNETT, C. W.
1940. THE RELATION OF VIRUSES TO PLANT TISSUES. Bot. Rev. 6: 427–473.

(6) Biebl, R.
1935. Die Wirkung der α-Bestrahlung auf Protoplasma und Chloroplasten. Protoplasma 24: [225]–257, illus.
(7) Böning, K.
1927. Die Mosaikkrankheit der Rübe. Forsch. auf dem Geb. Pflanzenkrank. u. Immunität im Pflanzenr. 3: [81]–128, illus.
(8) ———
1927. Die Mosaikkrankheit der Ackerbohne (Vicia faba L.). Ein Beitrag zu dem Mosaik der Papilionaceen. Forsch. auf dem Geb. Pflanzenkrank. u. Immunität im Pflanzenr. 4: [43]–111, illus.
(9) Clinch, P.
1932. Cytological studies of potato plants affected with certain virus diseases. Roy. Dublin Soc., Sci. Proc. 20: 143–172, illus.
(10) Cook, M. T.
1925. Histology and cytology of sugar cane mosaic. Porto Rico Dept. Agr. Jour. 9: 5–27, illus.
(11) ———
1926. The effect of mosaic on the content of the plant cell. Porto Rico Dept. Agr. Jour. 10: 229–238, illus.
(12) ———
1930. The effect of some mosaic diseases on cell structure and on the chloroplasts. Porto Rico Dept. Agr. Jour. 14: 69–101, illus.
(13) ———
1931. The effect of mosaic on cell structure and chloroplasts. Porto Rico Depts. Agr. Jour. 15: 177–181, illus.
(14) Dickson, B. T.
1922. Studies concerning mosaic diseases. Macdonald Col., McGill Univ. Tech. Bul. 2: 1–125, illus.
(15) Doolittle, S. P.
1920. The mosaic disease of cucurbits. U. S. Dept. Agr. Bul. 879, 69 pp., illus.
(16) Dunlap, A. A.
1931. Carbohydrate variations accompanying the mosaic disease of tobacco. Amer. Jour. Bot. 18: 328–336.
(17) Eckerson, S. H.
1926. An organism of tomato mosaic. Bot. Gaz. 81: 204–209, illus.
(18) Esau, K.
1933. Pathologic changes in the anatomy of leaves of the sugar beet, Beta vulgaris L. affected by curly top. Phytopathology 23: 679–712, illus.
(19) ———
1934. Ontogeny of phloem in the sugar beet (Beta vulgaris L.) Amer. Jour. Bot. 21: 632–644, illus.
(20) ———
1935. Ontogeny of the phloem in sugar beets affected by the curly-top disease. Amer. Jour. Bot. 22: 149–163, illus.
(21) ———
1938. Some anatomical aspects of plant virus disease problems. Bot. Rev. 4: 548–579.
(22) ———
1941. Phloem anatomy of tobacco affected with curly top and mosaic. Hilgardia 13: 437–490, illus.
(23) Faull, A. F.
1935. Elaioplasts in iris: a morphological study. Arnold Arboretum Jour. 16: 225–267, illus.
(24) Gicklhorn, J.
1932. Vorübergehende Formänderungen von Plastiden während der Plasmolyse. Protoplasma 15: [71]–89, illus.
(25) Goldstein, B.
1926. A cytological study of the leaves and growing points of healthy and mosaic diseased tobacco plants. Torrey Bot. Club Bul. 53: 499–599, illus.

(26) GRAINGER, J.
1933. THE MOVEMENT OF TOBACCO MOSAIC VIRUS IN ITS HOST. Ann. Appl. Biol. 20: 236–257, illus.
(27) ——— and Heafford, R. M.
1933. SOME EFFECTS OF THE ORDINARY TOBACCO MOSAIC UPON THE DEVELOPMENTAL ANATOMY OF THE HOST PLANT. Leeds Phil. and Lit. Soc. Proc. 2: 406–415, illus.
(28) GRATZY-WARDENGG, E.
1931. DEGENERATION VON CHLOROPLASTEN AN FARNPROTHALLIEN. Protoplasma 14: [52]–63, illus.
(29) GUILLIERMOND, A., MANGENOT, G., and PLANTEFOL, L.
1933. TRAITÉ DE CYTOLOGIE VÉGÉTALE. 1195 pp., illus. Paris.
(30) HEIN, I.
1926. CHANGES IN PLASTIDS IN VARIEGATED PLANTS. Torrey Bot. Club Bul. 53: 411–418, illus.
(31) HEITZ, E.
1936. UNTERSUCHUNGEN ÜBER DEN BAU DER PLASTIDEN. I. DIE GERICHTETEN CHLOROPHYLLSCHEIBEN DER CHLOROPLASTEN. Planta 26: [134]–163, illus.
(32) HOGGAN, I. A.
1927. CYTOLOGICAL STUDIES ON VIRUS DISEASES OF SOLANACEOUS PLANTS. Jour. Agr. Res. 35: 651–671, illus.
(33) HOLMES, F. O.
1928. ACCURACY IN QUANTITATIVE WORK WITH TOBACCO MOSAIC VIRUS. Bot. Gaz. 86: 66–81, illus.
(34) IWANOWSKI, D.
1903. ÜBER DIE MOSAIKKRANKHEIT DER TABAKSPFLANZE. Ztschr. f. Pflanzenkrank. 13: 1–41, illus.
(35) JOHANSEN, D. A.
1940. PLANT MICROTECHNIQUE. 523 pp., illus. New York and London.
(36) KNUDSON, L.
1936. HAS THE CHLOROPLAST A SEMIPERMEABLE MEMBRANE? (Abstract.) Amer. Jour. Bot. 23: 694.
(37) ———
1940. PERMANENT CHANGES OF CHLOROPLASTS INDUCED BY X RAYS IN THE GAMETOPHYTE OF POLYPODIUM AUREUM. Bot. Gaz. 101: 721–758, illus.
(38) KÜSTER, E.
1927. BEITRÄGE ZUR PHYSIOLOGIE UND PATHOLOGIE DER CHLOROPLASTEN. Protoplasma 2: [65]–79, illus.
(39) ———
1937. PATHOLOGIE DER PFLANZENZELLE. II. PATHOLOGIE DER PLASTIDEN. 152 pp., illus. Berlin.
(40) LEE, A. B.
1924. THE MICROTOMIST'S VADE-MECUM. A HANDBOOK OF THE METHODS OF MICROSCOPIC ANATOMY. Ed. 8, 594 pp., illus. Philadelphia. (Reprint.)
(41) LIEBALDT, E.
1913. ÜBER DIE WIRKUNG WÄSSERIGER LÖSUNGEN OBERFLÄCHENAKTIVER SUBSTANZEN AUF DIE CHLOROPHYLLKÖRNER. Ztschr. f. Bot. 5: [65]–113, illus.
(42) MATSUMOTO, T.
1922. SOME EXPERIMENTS WITH AZUKI-BEAN MOSAIC. Phytopathology 12: [295]–297, illus.
(43) MURAVIOV, V. P., ed.
1930. MOSAIC DISEASES OF THE SUGAR BEET. (Mozaichnye bolezni sakharnoi svekly.) U. S. S. R. Soiuzsakhara (Plant Breeding Dept., Union Sugar Concern) 286 pp., illus. Kiev.
(44) NELSON, R.
1932. INVESTIGATIONS IN THE MOSAIC DISEASE OF BEAN (PHASEOLUS VULGARIS L.). Mich. Agr. Expt. Sta. Tech. Bul. 118, 71 pp. illus.
(45) PETELER, K.
1939. AMÖBOIDE FORMVERÄNDERUNGEN DER DIATOMEEN-PLASTIDEN. Protoplasma 32: [9]–19, illus.
(46) ROBBINS, W. W.
1921. MOSAIC DISEASE OF SUGAR BEETS. Phytopathology 11: [349]–365, illus.

(47) ROLAND, G.
1936. RECHERCHES SUR LA JAUNISSE DE LA BETTERAVE ET QUELQUES OBSERVATIONS SUR LA MOSAÏQUE DE CETTE PLANTE. Sucr. Belge 55: 263–268, illus.
(48) SCHAFFNIT, E., and WEBER, H.
1927. ÜBER DAS VORKOMMEN VON INTRAZELLULAREN KÖRPERN IN DEN GEWEBEN MOSAIKKRANKER RÜBEN. Forsch. auf dem Geb. Pflanzenkrank. u. Immunität im Pflanzenr. 4: [23]–42, illus.
(49) SCHÜRHOFF, P. N.
1924. DIE PLASTIDEN. *In* Linsbauer, K. Handbuch der Pflanzenanatomie. Allgemeiner Teil: Cytologie. Bd. 1, 224 pp., illus. Berlin.
(50) SCHWARZ, W.
1928. ZUR ÄTIOLOGIE DER GEADERTEN PANASCHIERUNG. (I. MITTEILUNG.) Planta 5: [660]–680, illus.
(51) ———
1930. ÜBER DIE URSACHEN UND DAS ZUSTANDEKOMMEN DER PANASCHIERUNG BEI EINER FORM DER SELAGINELLA MARTENSII SPRING. FOL. VAR. Protoplasma 10: [427]–451, illus.
(52) SENN, G.
1908. DIE GESTALTS-UND LAGEVERÄNDERUNG DER PFLANZEN-CHROMATOPHOREN. 397 pp., illus. Leipzig.
(53) SHEFFIELD, F. M. L.
1933. THE DEVELOPMENT OF ASSIMILATORY TISSUE IN SOLANACEOUS HOSTS INFECTED WITH AUCUBA MOSAIC OF TOMATO. Ann. Appl. Biol. 20: 57–69, illus.
(54) SIROTINA, M.
1932. A CYTOLOGICAL STUDY OF MOSAIC DISEASE OF SUGAR BEET. (TSITOLOGICHESKOE IZUCHENIE MOZAIKI SAKHARNOI SVEKLY.) Nauk. Zapiski Tsukr. Prom. Ukrain. Nauk.-Dosl. Inst. Tsukr. Prom. [Sci. Contr. Sugar Ind. Ukrain. Res. Inst. Sugar Ind.] 9 (24): 195–216. Illus. [In Russian. English summary.]
(55) SMITH, F. F.
1926. SOME CYTOLOGICAL AND PHYSIOLOGICAL STUDIES OF MOSAIC DISEASES AND LEAF VARIEGATIONS. Mo. Bot. Gard. Ann. 13: 425–484. Illus.
(56) SOROKIN, H.
1927. PHENOMENA ASSOCIATED WITH THE DESTRUCTION OF THE CHLOROPLASTS IN TOMATO MOSAIC. Phytopathology 17: 363–379, illus.
(57) STONE, W. E.
1942. EFFECTS OF SOME MILD FORMS OF MOSAIC ON POTATO AND A FEW OTHER PLANTS. Jour. Agr. Res. 65: 195–207, illus.
(58) TOWNSEND, C. O.
1915. SUGAR-BEET MOSAIC. Science 42: 219–220.
(59) WEIER, T. E.
1933. NEUTRAL RED STAINING IN THE PROTONEMA OF POLYTRICHUM COMMUNE. Amer. Jour. Bot. 20: 431–439, illus.
(60) WEIER, [T.] E.
1936. THE STRUCTURE OF THE NON-STARCH-CONTAINING BEET CHLOROPLAST. Amer. Jour. Bot. 23: 645–652, illus.
(61) ———
1938. THE STRUCTURE OF THE CHLOROPLAST. Bot. Rev. 4: 497–530, illus.
(62) ———
1938. VIABILITY OF CELLS CONTAINING CHLOROPLASTS WITH AN OPTICALLY HOMOGENEOUS OR GRANULAR STRUCTURE. Protoplasma 31: [346]–350.
(63) ———
1939. THE MICROSCOPIC APPEARANCE OF THE CHLOROPLAST. Protoplasma 32: [145]–152.
(64) WIELER, A.
1936. ÜBER DEN BAU DER CHLOROPHYLLKÖRNER. Protoplasma 26: [295]–311, illus.
(65) WOODS, A. F.
1900. INHIBITING ACTION OF OXIDASE UPON DIASTASE. Science 11: 17–19.
(66) ———
1902. OBSERVATIONS ON THE MOSAIC DISEASE OF TOBACCO. U. S. Bur. Plant Indus. Bul. 18, 24 pp., illus.

599130—44——3

REACTION OF LACTUCA SPECIES TO THE ASTER YELLOWS VIRUS UNDER FIELD CONDITIONS [1]

By Ross C. Thompson

Horticulturist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture

INTRODUCTION

The loss to lettuce growers attributable to the aster yellows virus in certain localities in the eastern part of the United States has amounted to as much as 30 percent of the crop in some seasons. The increasing seriousness of this disease has emphasized the need for a more satisfactory means of control than is at present available. In the hope of determining whether genetic factors for resistance to the aster yellows virus that might be of value in breeding resistance into the important commercial varieties could be located in any of the numerous species and varieties of lettuce, investigations were initiated. In 1935 a large collection of varieties of cultivated lettuce from both domestic and foreign sources was started at the Bureau of Plant Industry Station, Beltsville, Md. These were tested under field conditions to determine their reaction to the aster yellows virus. After several years of field testing it was concluded that none of the varieties of cultivated lettuce carry factors that are likely to be of value in breeding for resistance to this disease. Attention was then turned to the wild species of *Lactuca*. As many wild forms as could be obtained were collected and tested under field conditions for their reaction to the aster yellows virus.

Yellows of aster (*Callistephus chinensis* (L.) Nees) was described by Smith (*7*),[2] who suggested that similar symptoms in other Compositae might be due to the same cause. According to Kunkel (*2*, *3*), the aster yellows virus is spread from plant to plant almost entirely by a single species of leafhopper (*Macrosteles divisus* (Uhl.)). He listed some 170 species of plants to which the virus was transmitted by *M. divisus*. Numerous other host plants have been added to the list. Some of these are wild forms that provide overwintering hosts for the leafhopper; from them the virus is spread to cultivated plants.

Probably the first report of aster yellows in lettuce was made by Carpenter (*1*), who indicated it to be the cause of considerable loss to lettuce growers in the Rio Grande Valley of Texas. Severin (*5*) presented data which indicated that the strain of aster yellows virus found in California differed from the eastern strain. He (*6*) found a number of varieties of lettuce to be susceptible to the California strain. Linn (*4*) listed 23 varieties of lettuce that were susceptible to the eastern strain on Staten Island, N. Y. It is assumed that

[1] Received for publication March 10, 1943. The writer is indebted to the Division of Plant Exploration and Introduction for many of the species, varieties, and strains of *Lactuca* used in these investigations.

[2] Italic numbers in parentheses refer to Literature Cited, p. 124.

the eastern strain of the aster yellows virus described by the above-mentioned workers is the one involved in the study of *Lactuca* reported herein, but no effort has been made to verify the assumption.

MATERIALS

AMERICAN GARDEN VARIETIES

The following varieties of *Lactuca sativa* L. from American sources were grown over a period of several years, and notes were made on their reaction to aster yellows: Asparagus, Batavian Bath Cos, Bibb, Big Boston, Black-Seeded Simpson, Brown Batavian, California Cream Butter, Chavigne, Crisp as Ice, Dark Green Cos, Deacon, Deer's Tongue, Density, Denver Market, Early Curled Simpson, Giant Summer, Golden Queen, Grand Rapids, Green Madeira Winter, Green Province Cos, Hanson, Hardy Green Winter, Hardy Red Winter, Hubbards Market, Iceberg, Imperial C, Imperial D, Imperial F, Imperial 44, Imperial 152, Imperial 615, Imperial 847, Imperial 850, Malta, Mammoth Black-Seeded Butter, Marvel, Maximum, May King, Mignonette, Milly, New York Regular, New York 12, New York 515, Paris White Cos, Prize Head, Salamander, Speckled Dutch Butter, Tennisball, Tom Thumb, Unrivalled, and Wayahead.

EUROPEAN GARDEN VARIETIES

The following European varieties of lettuce obtained from Vilmorin-Andrieux & Cie., Paris, France, were included among the varieties studied: Batavian Bath, Brown Batavian, Brown Genoa, Brown Stolid, Brown Stonehead, Brown Winter, Giant Summer, Green Madeira Winter, Green Province Cos, Hardy Green Winter, Hardy Red Winter, Madeira Large Winter, Market Gardener's Red, Marvel, Milly, Red Edged Victoria, and White Batavian.

INTRODUCTIONS

In addition to the varieties just listed a large number of strains of *Lactuca* obtained from various foreign sources through the Division of Plant Exploration and Introduction have been grown and their reactions to the aster yellows virus have been noted. These strains had the following P. I. numbers: 113351 to 113353, 114033, 115189 to 115191, 115476, 115591, 115592, 115943, 116517, 116933, 117208 to 117216, 117892 to 117895, 118306, 118307, 118309 to 118317, 118319 to 118321, 118601, 118683, 118684, 119773, 120932 to 120938, 120940 to 120949, 120951 to 120966, 121935 to 121938, 121940, 124124, 127335, 131425, 133493, 133494, 134205, 134206, 138584 to 138598, 138912, 140392 to 140402, 141680 to 141683, 141961, 141962, 142863 to 142872. Many of these introduced strains were obviously American and European named varieties or very similar strains, but no attempt was made to identify them.

WILD SPECIES

The following *Lactuca* species obtained from the sources indicated were included in the tests in 1939, 1940, 1941, and 1942:

Lactuca altaica Fisch. and Mey., from Egypt, through Division of Plant Exploration and Introduction.

L. bourgaei (Boiss.) Irish and Taylor, from England, through Division of Plant Exploration and Introduction.

L. canadensis L., collected in Maryland.
L. floridana (L.) Gaertn., collected in Maryland.
L. graminifolia Michx., from South Carolina, through the late J. B. Norton.
L. indica L., from China, through G. L. Stebbins, Jr.
L. marschallii Stebbins, from Sweden, through G. L. Stebbins, Jr.
L. muralis (L.) Fresen., from Sweden, through G. L. Stebbins, Jr.
L. perennis L., from Wayside Gardens, Mentor, Ohio.
L. raddeana Maxim., from Union of Soviet Sociali t Republics, through Division of Plant Exploration and Introduction.
L. saligna L., from Ohio, through G. L. Stebbins, Jr.
L. saligna L. (P. I. No. 120950), from Turkey, through Division of Plant Exploration and Introduction.
L. serriola L., collected in Rock Creek Park, D. C.
L. serriola L., (P. I. No. 114512), from Sweden, through Division of Plant Exploration and Introduction.
L. serriola L. (P. I. No. 114535), from Afghanistan, through Division of Plant Exploration and Introduction.
L. serriola L. (P. I. No. 125819), from Afghanistan, through the Division of Plant Exploration and Introduction.
L. spicata (Lam.) Hitchc., collected in Virginia.
L. squarrosa (Thunb.) Miq., from Union of Soviet Socialist Republics, through Division of Plant Exploration and Introduction.
L. squarrosa (Thunb.) Miq. (*L. laciniata* (Houtt.) Makino), from Asia, through Division of Plant Exploration and Introduction.
L. tatarica (L.) C. A. Mey., from Cap d'Antibes, France, through G. L. Stebbins, Jr.
L. virosa L. (annual form), from Surrey, England, through Division of Plant Exploration and Introduction.
L. virosa L. (biennial form), from Stockholm, Sweden, through Division of Plant Exploration and Introduction.

METHODS

The tests for response to the aster yellows virus were all made under field conditions. The seed was planted in wooden flats in a greenhouse, and when the seedlings were large enough to transplant they were removed from the flats and set in 5-ounce paper drinking cups. The plants were later transplanted to the field where they were exposed to natural infection. The large number of strains to be tested made it impractical to attempt controlled inoculations.

Many of the varieties of cultivated lettuce were grown for only one season. If a variety showed a fairly large number of yellows-infected plants, it was considered susceptible and probably of no value for breeding for resistance and was eliminated from future tests. If the number of infected plants was quite small in the first planting, the variety was grown again before eliminating it.

RESULTS

The loss from aster yellows in spring lettuce has always been very high in plantings at the Bureau of Plant Industry Station, Beltsville, Md. Even in years when the percentage of affected plants has been lowest, field tests for varietal response to the virus have been satisfactory. A high percentage of the plants of susceptible varieties have always been diseased when the crop has been started early.

CULTIVATED VARIETIES

It was at first thought that some of the plants of the cultivated varieties that did not develop yellows might be resistant. Many such plants have been tested for resistance in succeeding generations,

but in no case have they shown indication of resistance. In 1937, a year of high field infection, approximately 10,000 plants of many cultivated varieties and strains were checked throughout the season for plants that escaped infection. Many of the plants were lost during the summer from causes other than aster yellows. At the end of the season (late August) only 1 plant that did not show infection with yellows survived to produce seed. Seed of this plant was saved, and 200 plants were grown in the field the following year. Some of these escaped the yellows, but none of them showed any indication of resistance in later generations.

Because of the great number of leafhoppers present in the fields where these tests were conducted, it has often been difficult to see how any plants could escape inoculation. As the season advanced the number of infected plants increased, greatly magnifying the chances for the spread of the virus. However, numerous subsequent tests of disease-free plants of the cultivated varieties indicate that they escaped inoculation rather than that they were resistant.

That the yellows-free individuals are plants that have somehow escaped infection and are not resistant is as would be expected, since natural crossing in lettuce is limited and most varieties are highly homozygous. For this reason, it seems fairly safe to assume that the appearance of even a small percentage of diseased plants in a population is sufficient to permit the variety to be classed as susceptible. Single 50-foot rows of many varieties were enough to show their susceptibility; however, as many as 200 plants of some varieties were grown.

All 200 lots of cultivated lettuce obtained from American and European seedsmen and from various foreign sources, through the Division of Plant Exploration and Introduction, were found to be susceptible to the aster yellows virus. Although the varieties tested do not exhaust the list of known varieties and strains of cultivated lettuce, the number of strains included makes doubtful the finding of genetic factors for resistance to the aster yellows virus in *Lactuca sativa*.

WILD SPECIES

Preliminary tests of wild species indicated that some of them were much more resistant to the virus than the cultivated varieties. A replicated plot test was set up with the intention of growing 4 replicated plots of 25 plants of each species. Such tests were carried out over the 4-year period, 1939 to 1942. A few species were added to the list after the tests were started; hence they were not grown all 4 years. Some of the species were found to be especially susceptible to diseases other than aster yellows. In some cases an entire replication of certain species was lost through attacks of some of these diseases. Heavy rains in some seasons and severe drought in others resulted in damage to and loss of plants. As a consequence of these and other factors there were great differences both in the number of plants per plot and in the number of plants of different species.

In the meantime hybridization studies in which the genetic compatibility of many of these wild species with *Lactuca sativa* was investigated were reported by Thompson (*8*) and Thompson, Whitaker, and Kosar (9). The studies indicated that, because of genetic incompatibility, many of the wild species could be of no value for

breeding even though they might be highly resistant to aster yellows.

The results from the field tests of wild species are given in table 1 in terms of percentage of plants showing symptoms of aster yellows.

TABLE 1.—*Summary of data on reaction of some species of Lactuca to the aster yellows virus*

Species of *Lactuca*	1939		1940		1941		1942		Total	
	Plants tested	Plants with yellows	Plants tested	Plants with yellows	Plants tested	Plants with yellows	Plants tested	Plants with yellows	Plants tested	Plants with yellows
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
L. altaica	45	42.22	93	22.58	99	11.11	76	11.84	313	19.17
L. bourgaei	62	0	81	0	83	0	35	0	261	0
L. canadensis	93	7.53	76	5.26	91	6.79	83	6.02	343	6.71
L. floridana	92	8.70	87	5.75	97	13.4	89	7.87	365	9.04
L. graminifolia	95	6.32	93	2.15	95	12.63	73	6.85	356	7.02
L. indica	85	34.12	98	7.14	83	20.48	79	16.46	345	19.13
L. marschallii	------	------	75	0	50	0	99	0	224	0
L. muralis	46	76.10	93	13.98	77	32.47	61	31.15	277	33.21
L. perennis	79	49.37	90	6.67	73	20.55	85	8.24	327	20.49
L. raddeana	87	5.75	95	3.16	71	9.86	95	8.42	348	6.61
L. saligna (native)	99	9.09	94	8.51	99	7.07	87	4.60	379	7.39
L. saligna (P. I. No. 120950)	100	5.00	91	4.40	94	2.13	91	3.30	376	3.72
L. serriola (local)	------	------	86	5.81	65	3.08	15	6.67	166	4.82
L. serriola (P. I. No. 114512)	------	------	------	------	88	5.68	88	1.14	176	3.41
L. serriola (P. I. No. 114535)	98	29.59	97	8.24	91	27.47	90	12.22	376	19.41
L. serriola (P. I. No. 125819)	86	59.30	99	17.17	97	27.84	88	28.41	370	32.43
L. spicata	99	18.18	92	6.52	87	17.24	86	5.81	364	12.09
L. squarrosa	------	------	87	17.24	90	16.67	------	------	177	16.95
L. squarrosa (*L. laciniata*)	89	98.87	96	36.46	92	64.13	95	49.47	372	61.56
L. tatarica	90	0	78	0	81	0	91	0	340	0
L. virosa (annual)	99	79.80	89	48.31	93	61.29	97	61.86	378	63.23
L. virosa (biennial)	------	------	95	31.58	91	60.44	87	48.88	273	46.52

Since the loss of plants from various causes introduced great variation in the field plot data and all but two of the species have been eliminated as possible breeding material because of their genetic incompatibility with *Lactuca sativa*, no attempt has been made to use the data to indicate the relative significance of the differences in the response of the species to the aster yellows virus.

Three of the 22 species and strains of wild lettuce included in these tests (*Lactuca bourgaei*, *L. marschallii*, and *L. tatarica*) seemed immune to the virus. No plants of any of these 3 species showed symptoms of yellows in any of the replications in any of the years in which they were tested. Thompson, Whitaker, and Kosar (*9*) and Thompson (*8*) found that these 3 species were genetically incompatible with *Lactuca sativa;* hence, they are of no direct value in breeding for yellows resistance in cultivated lettuce.

Four 17-chromosome species (*Lactuca canadensis*, *L. floridana*, *L. graminifolia*, and *L. spicata*) showed comparatively low percentages of diseased plants. These 4 have also been found by the above-named writers to be genetically incompatible with *L. sativa;* hence, they are of no direct value as breeding material. The appearance of yellows in some of these species is not proof of complete susceptibility as is the presence of diseased plants in cultivated varieties, since some of the former are not entirely homozygous and some degree of resistance

may be present in some plants even though many plants succumb to the virus.

Lactuca squarrosa (*L. laciniata*) and *L. virosa* had consistently high percentages of diseased plants and are apparently very susceptible to the virus. These two species have also been found to be genetically incompatible with *L. sativa*.

Of the 22 species and strains tested only the strains of *Lactuca serriola* and *L. saligna* indicate value as breeding material and there is some question whether any use can be made of *L. saligna*. The data indicate that 2 strains of *L. serriola*, the local strain and P. I. No. 114512, and the 2 strains of *L. saligna* may be of value in breeding for aster yellows resistance in *L. sativa*. The *L. serriola* strains cross readily with *L. sativa* and give fertile progenies. So far it has not been possible to cross the *L. saligna* strains with *L. sativa*. The *L. saligna* strains have both been crossed with *L. serriola*, and partly fertile hybrids have resulted. It may be possible to use the *L. saligna* strains in breeding by transferring the desired hereditary factors to *L. serriola* and then through the hybrid to *L. sativa*. So far the attempt to make a cross between the *L. serriola* × *L. saligna* hybrid and *L. sativa* has not been successful. An important point not yet investigated is whether the low disease incidence of these *L. serriola* and *L. saligna* strains is due to direct resistance to the virus or to some peculiarity of the plant that repels the vector. The consistently low percentages of diseased plants occurring in these 4 strains indicate that they have some capacity for resisting virus infection and may be of value in breeding for resistance to the aster yellows virus.

SUMMARY

Two hundred varieties and strains of cultivated lettuce and 22 wild species and strains of *Lactuca* were tested under field conditions for their reaction to the aster yellows virus. All the strains of the cultivated varieties indicated susceptibility to the virus. The wild species varied greatly in their susceptibility to infection under field conditions.

Lactuca tatarica, *L. bourgaei*, and *L. marschallii* seemed to be immune to the aster yellows virus, but they have no direct value in breeding for resistance, since they have been found to be genetically incompatible with the cultivated varieties.

Two strains of *Lactuca serriola* and two strains of *L. saligna* showed marked ability to escape infection. These species may prove to be of value in breeding for resistance in cultivated lettuce; especially the *L. serriola* forms, which cross readily with cultivated lettuce.

The 17-chromosome species (*Lactuca canadensis*, *L. spicata*, *L. graminifolia, and L. floridana*) as a group indicated some ability to escape infection, but they offer no promise as material for breeding for aster yellows resistance, as they have been found to be genetically incompatible with the cultivated form.

Lactuca virosa and *L. squarrosa* (*L. laciniata*) are highly susceptible as was indicated by a consistently high percentage of infected plants.

LITERATURE CITED

(1) Carpenter, C. W.
1916. The Rio Grande disease of lettuce. Phytopathology 6: 303–305, illus.

(2) Kunkel, L. O.
1926. Studies on aster yellows. Amer. Jour. Bot. 13: 646–705, illus.

(3) KUNKEL, L. O.
1931. STUDIES ON ASTER YELLOWS IN SOME NEW HOST PLANTS. Boyce Thompson Inst. Contrib. 3: 85–123, illus.
(4) LINN, M. B.
1940. THE YELLOWS DISEASE OF LETTUCE AND ENDIVE. N. Y. (Cornell) Agr. Expt. Sta. Bul. 742, 33 pp., illus.
(5) SEVERIN, H. H. P.
1934. EXPERIMENTS WITH THE ASTER-YELLOWS VIRUS FROM SEVERAL STATES. Hilgardia 8: 305–325, illus.
(6) ———
1929. YELLOWS DISEASE OF CELERY, LETTUCE, AND OTHER PLANTS TRANSMITTED BY CICADULA SEXNOTATA (FALL.) Hilgardia 3: 543–582, illus.
(7) SMITH, R. E.
1902. GROWING CHINA ASTERS. Mass. (Hatch) Agr. Expt. Sta. Bul. 79, 26 pp., illus.
(8) THOMPSON, R. C.
1943. FURTHER STUDIES ON INTERSPECIFIC GENETIC RELATIONSHIPS IN LACTUCA. Jour. Agr. Res. 66: 41–48, illus.
(9) ——— WHITAKER, T. W., and KOSAR, W. F.
1941. INTERSPECIFIC GENETIC RELATIONSHIPS IN LACTUCA. Jour. Agr. Res. 63: 91–107, illus.

DIFFERENTIAL EFFECTS OF TEMPERATURE ON THE DEVELOPMENT OF THE BEET LEAFHOPPER[1, 2]

By F. H. HARRIES

Associate entomologist, Truck Crop and Garden Insect Investigations, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, United States Department of Agriculture

INTRODUCTION

The maturation and time of movement of the spring generation of beet leafhoppers (*Eutettix tenellus* (Bak.)) from desert breeding areas to the cultivated sections and the seasonal sequence and number of the generations produced annually are governed largely by temperature. Since it was desirable to know more definitely the direct effect of temperature and changes in temperature on development of the insect during investigation of these and other problems of seasonal development, maturation of the different stages of the leafhopper was studied under controlled conditions.

Investigations by various workers (*6,8,9,10,13*)[3] have indicated that insect development may occur more rapidly at varying temperatures than would be expected from results obtained at constant temperatures. This effect has usually been attributed to a stimulus produced by temperature changes or, conversely, to an inhibitory influence of the unnatural exposure to the same temperature during the entire period of development. With few if any exceptions, effects of temperature on the rate of insect development have not been shown directly, but have been evaluated on the basis of end results indicated by index values taken as reciprocals of the time required to complete definite stages of metamorphosis. In this method it is assumed that at different temperatures the same portions of development require equal fractions of the total time necessary for completion of development.

There is evidence, however, that two or more growth phases unequally affected by different temperatures may occur within the same stage of development. This was shown by Crozier (*8*) from data by Titschack (*15*) on the pupal period of *Tineola bisselliella* (Hum.) and by Bliss (*2*) on the prepupa of *Drosophila melanogaster* Meig. Brown (*5*), analyzing the same data in conjunction with further studies on the development of a cladoceran at different temperatures, concluded that, in accordance with the nature of the equation proposed by Crozier, the analytical expression used to describe growth must have at least two velocity constants unequally affected by different temperatures. Powsner (*14*) showed that the temperature coefficient differed in successive portions of the embryonic period of *D. melanogaster*, and other data on the pupal stage of the same insect by Ludwig and Cable (*11*) support this interpretation.

[1] Received for publication April 22, 1943.
[2] The writer is indebted to J. R. Douglass and W. C. Cook for supervision during the present study and to P. N. Annand for other assistance and suggestions.
[3] Italic numbers in parentheses refer to Literature Cited, p. 135.

When eggs of the beet leafhopper were exposed alternately for 12-hour periods to two temperatures, development was always completed in less time than would be expected on the basis of the time required for complete development at each constant temperature (*9*). This difference in time represents a stimulating effect of change in temperature provided the course of development is modified solely by the time required for completion of the entire stage. To determine if any factor besides time is involved, the experiments described in this paper were initiated.

METHODS

The eggs and nymphs were obtained from a supply of adults that had been collected in the fall and kept in the laboratory during the winter. Small sugar-beet plants selected for uniformity of size and color were used as hosts. They were grown in the greenhouse in 4-inch pots and were used when about 4 to 6 inches high.

Eggs were obtained by placing gravid females on the plants in small cages designed to fit snugly around the petioles. After 12 hours the cages were removed and the portions of the plant in which the eggs were embedded were marked with ink. Toward the end of embryonic development the cages were again placed over the petioles to confine the young nymphs. In studies of the nymphal stage the insects were confined in cages placed over entire plants. These cages were about 6 inches high and 3 inches in diameter, and were made of thin, fine-meshed cloth over a frame of celluloid ribs. A metal band attached to the frame was pressed into a layer of sand covering the soil around the potted beets. Both types of cages were designed to provide a maximum circulation of air, and thus to minimize any effect of transpiration on temperature and atmospheric moisture within the cages.

The insects were exposed in cabinets [4] in which the temperature was controlled within 1° F. and the relative humidity was kept between 49 and 51 percent. Continuous records were obtained with hygrothermograph recorders, and temperature and humidity within the cages were checked with a thermometer and a small dew-point tube. Insects were subjected to a change in temperature during development by transfer from one chamber to another. Some groups of eggs and nymphs were transferred from a higher to a lower temperature and others from a lower to a higher temperature. Direct observations of the growth of nymphs were made daily by weighing groups of 50 on an analytical balance.

RESULTS AND DISCUSSION

The effect of exposure of the beet leafhopper at one temperature for the first part of the egg or nymphal period and at another temperature for the remainder of the stage is shown in table 1. The duration observed when the temperature was changed is compared with that expected on the basis of the durations observed when the insects were exposed for the entire stage at each of the temperatures involved.

[4] ANNAND, P. N., and HARRIES, F. H. TEMPERATURE-HUMIDITY CONTROLLED CABINETS FOR THE STUDY OF INSECTS U. S. Bur. Ent. and Plant Quar. ET-159, 6 pp., illus. 1940. [Processed.]

TABLE 1.—*Duration of the egg and nymphal stages of the beet leafhopper when exposed for different periods to one temperature before being allowed to complete development at another temperature*

EGG STAGE

Temperature° F.		Individuals hatched or reared	Duration of exposure to first temperature	Duration of stage			Relative difference in duration [1]
First	Second			Observed	Expected	Difference	
		Number	*Days*	*Days*	*Days*	*Days*	*Percent*
90	65	603	0.0	6.27±0.02			
65	90	280	.0	26.35± .06			
90	65	135	1.0	21.63± .08	[2] 23.15	−1.52	−5.8
		110	1.5	20.12± .09	21.54	−1.42	−5.4
		219	2.0	18.12± .06	19.94	−1.82	−6.9
		287	2.5	16.69± .08	18.34	−1.65	−6.3
		280	3.0	14.86± .06	16.74	−1.88	−7.1
		86	3.5	13.17± .07	15.14	−1.97	−7.5
		158	4.0	11.45± .08	13.54	−2.09	−7.9
		177	4.5	9.73± .05	11.94	−2.21	−8.4
		100	5.0	8.24± .09	10.34	−2.10	−8.0
65	90	171	4	9.28± .02	9.32	−.04	−.6
		169	5	10.11± .03	10.08	.03	.5
		244	10	13.61± .03	13.89	−.28	−4.5
		392	12	15.42± .04	15.41	.01	.2
		158	15	17.81± .04	17.70	.11	1.8
		236	20	22.20± .02	21.51	.69	11.0

NYMPHAL STAGE

90	75	423	0	14.50±0.07			
75	90	463	0	27.50+ .12			
90	75	156	3	25.08± .22	24.81	.27	1.0
		412	6	21.49+ .13	22.12	−.63	−2.3
		486	9	17.87± .13	19.43	−1.56	−5.7
		239	12	16.78± .19	16.74	.04	.1
75	90	226	5	17.51± .08	16.86	.65	4.5
		146	10	20.08± .13	19.23	.85	5.9
		158	15	23.02± .10	21.59	1.43	9.9
		226	20	26.01± .10	23.95	2.06	14.2

[1] Difference in duration of stage expressed as percent of time required for development when exposed to the second temperature only.

[2] Expected duration in this case $=1+\frac{5.27}{6.27}(26.35)$.

When the change was from the higher to the lower temperature, the observed duration of development was usually shorter than the expected duration. Although only one change in temperature was made, some of the data show a decrease in duration of the stage comparable with that produced when temperature changes were made twice daily. On the other hand, when the change was reversed the duration was usually longer than expected. These differences seemed to increase as the duration of exposure at the first temperature increased. Corresponding differences associated with increases and decreases in temperature were not well correlated, but this could probably be expected, since the values are especially subject to experimental error in being dependent on the accuracy of three different means, the interval required for complete development at each of the two constant temperatures, and the interval required when the change was made from one constant temperature to another.

From such results it may be concluded that the relative durations of successive phases of development within the egg and nymphal stages differ at different temperatures. For example, the percentage of growth attained after half the time required for completion of the stage at a given temperature may be more, or less, than that reached

after half the time necessary at a different temperature, and a change from one temperature to another may result in apparent differences in developmental time without actually exerting a stimulating or inhibiting effect.

The progress of nymphal development at different temperatures is shown more directly by the daily weights given in table 2. These determinations are averages for a large number of nymphs, but since the insects were weighed in groups of 50, calculation of the standard errors was precluded by the limited number of samples.

TABLE 2.—*Average daily weights of the beet leafhopper during nymphal development at different constant temperatures and 50 percent relative humidity* [1]

Days after hatching	100° F.		90° F.		85° F.		80° F.		75° F.	
	Insects	Average weight	Insects	Average weight	Insects	Average weight	Insects	Average weight	Insects	Average weight
	Number	*Milligram*	*Number*	*Milligram*	*Number*	*Milligrams*	*Number*	*Milligrams*	*Number*	*Milligrams*
1	350	0.047	300	0.043	400	0.043	450	0.042	400	0.040
2	350	.060	350	.053	350	.051	400	.051	400	.045
3	350	.084	400	.067	350	.058	500	.055	450	.048
4	350	.101	400	.084	400	.067	450	.065	450	.054
5	350	.157	400	.126	350	.086	400	.078	400	.061
6	350	.202	500	.168	400	.104	350	.100	400	.069
7	250	.280	450	.198	350	.143	400	.123	500	.082
8	350	.389	400	.279	400	.174	400	.153	500	.090
9	350	.481	500	.335	400	.208	600	.189	600	.110
10	350	.589	550	.448	350	.273	350	.228	550	.133
11	350	.701	650	.592	350	.353	450	.256	550	.154
12	350	.800	500	.680	400	.397	450	.319	550	.173
13	350	.828	550	.835	350	.527	350	.342	500	.194
14	140	.932	260	.994	350	.619	450	.417	600	.215
15					300	.740	350	.482	550	.234
16					300	.814	350	.590	550	.279
17					180	1.004	350	.725	650	.313
18							250	.791	550	.364
19							250	.906	550	.412
20							200	1.043	550	.444
21									400	.544
22									450	.622
23									600	.658
24									550	.739
25									500	.868
26									400	.913
27									250	1.064
Average age when becoming adult (days)	13.84		14.5		17.39		20.48		27.48	

[1] The last weight in each column is that of the newly developed adult.

In figure 1 the daily course of growth is shown to define a typical sigmoid curve, in which the growth rate increases during the major part of the period and then decreases as the adult state is approached. The ratio of daily increase in weight, or the relative rate of growth, may be shown by plotting the logarithms of nymphal weight against time, as in figure 2. A linear distribution of the logarithms of successive weights would indicate a constant value for the relative rate of growth. The entire curve for each temperature is not a straight line, however, but appears to consist of linear segments of different slopes, which indicate that growth occurred at different constant rates in successive periods. These segments probably represent the five nymphal instars. With linear measurements of the mouth parts

of *Drosophila melanogaster,* Alpatov (*1*) showed that the growth curve consisted of three distinct segments when based on larvae of the same age, and that these periods were delineated as three com-

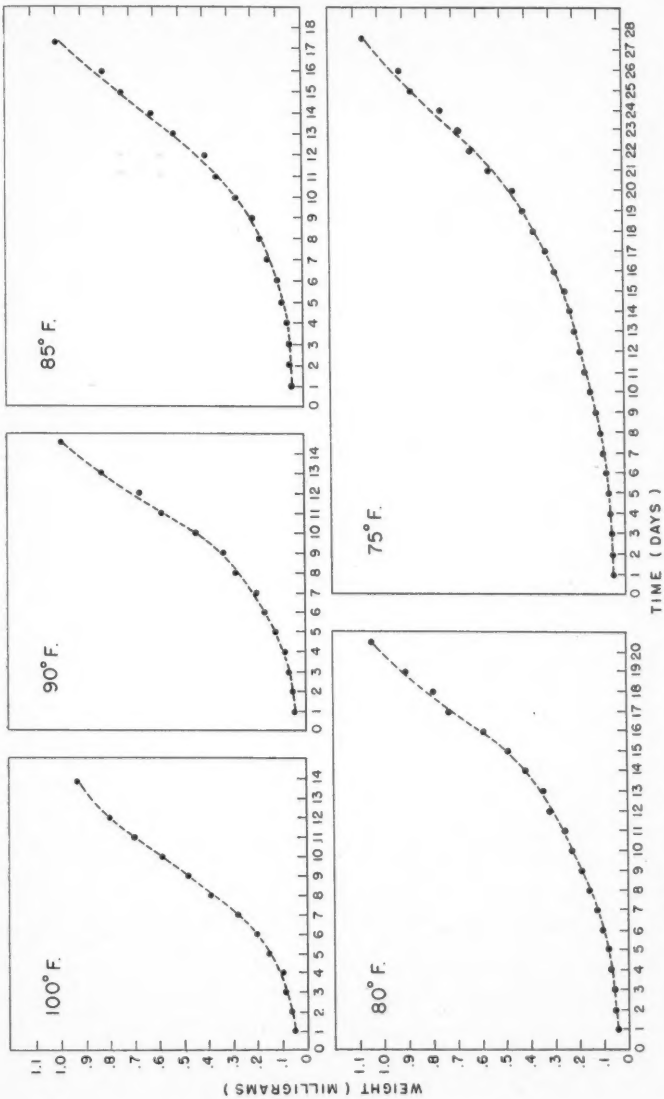


FIGURE 1.—Daily weights of nymphs of the beet leafhopper when reared at constant temperatures.

plete growth cycles when data on the different instars were plotted separately. Similar S-shaped curves were shown by Brindley (*4*) for larval growth of *Ephestia kuehniella* Zell. and *Tribolium confusum*

Duv. at a constant temperature, but the 3-day intervals at which the weight increments were determined were evidently too long to show possible differences in the rate of growth in different instars.

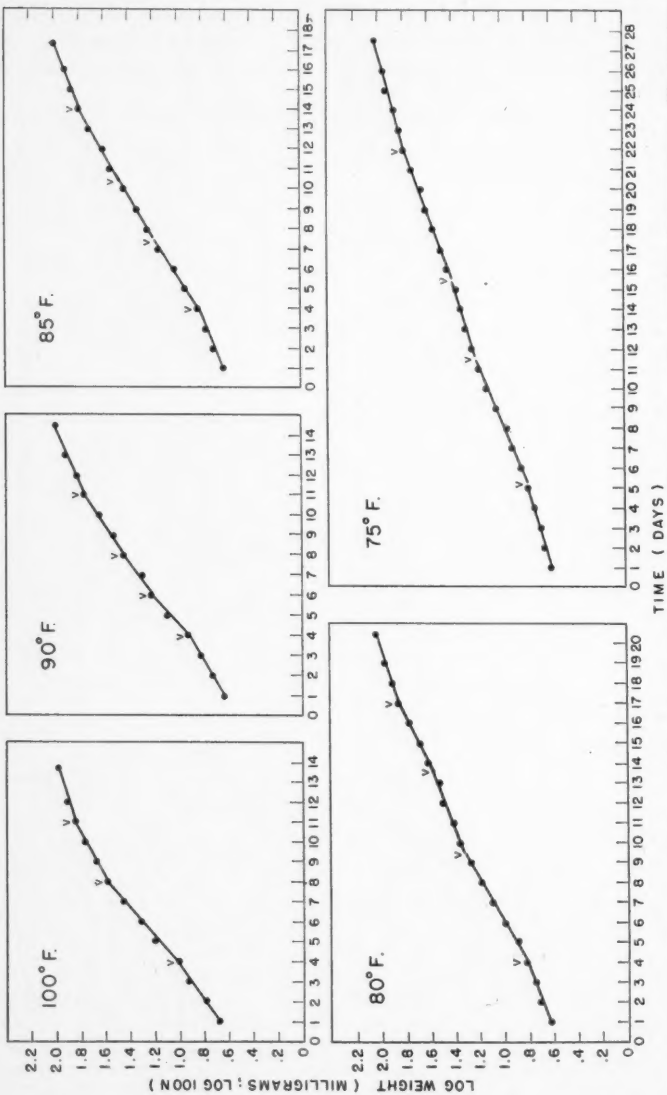


FIGURE 2.—Logarithms of daily weights of nymphs of the beet leafhopper reared at constant temperatures, illustrating changes in the growth ratio as development proceeds. Different curve segments are fitted to the equation $\log y = \log A + bx \log e$ by the method of least squares.

A comparison of the time course of growth at different temperatures is shown in figure 3, where the time factor is expressed in percentage of the total time required for development and the weight

factor in percentage of the adult weight. These curves, which are based on figures calculated from the regression lines of figure 2, show distinct divergence in most cases with differences of only 5° F., and the divergence becomes greater with greater differences in temperature. The fact that the curves do not coincide throughout shows that the relative rate of growth at different periods varies with the temperature, as was demonstrated by data in table 1 on the time

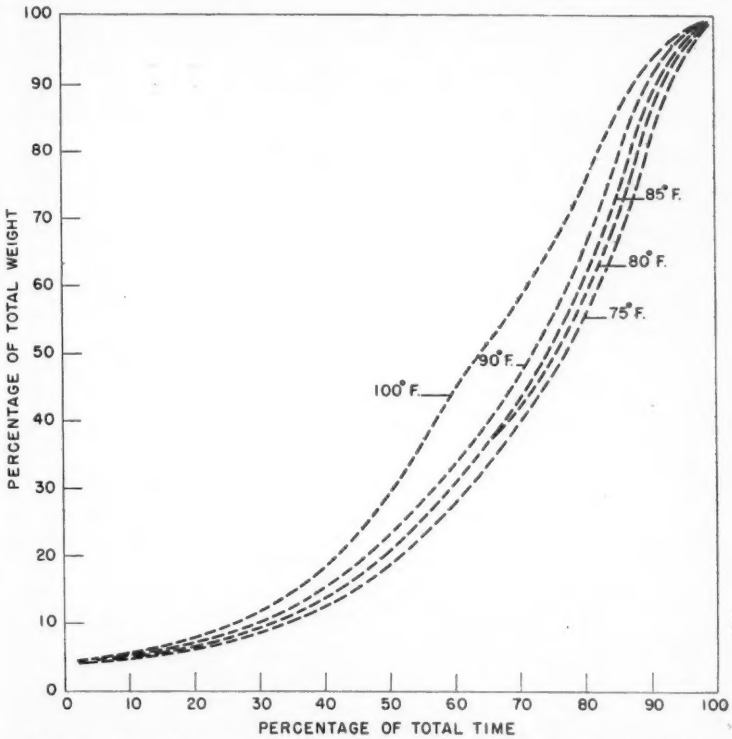


FIGURE 3.—Nymphal-growth curves at different temperatures, in which percentage of the adult weight is plotted against the percentage of the time required for development.

required for nymphal development in successive exposures to two different temperatures.

Regardless of the acceleration produced by changes in temperature, the nature of the growth curves makes it impossible to compute accurately the degree of acceleration from data on the duration of the entire stage at constant temperatures. Crozier (*8*) has shown that part of the difference in time resulting from a change from one temperature to another may be retained following a transfer back to the first temperature. It is uncertain how much of the apparent acceleration due to alternate 12-hour exposures to different tempera-

599130—44——4

tures (*9*) may be accounted for in this way, since differences in time resulting from changes in temperature would seem to be rather closely compensating in opposite transfers. In the same way effects of diurnal changes and alternate variations in temperature under natural conditions would tend to be compensating. In view of the effects of successive exposures to two temperatures shown in table 1, the greatest apparent acceleration or deceleration would evidently occur in successive periods of cool and warm weather and during the spring and fall when differences in daily mean temperatures are most pronounced. The relatively more rapid development at lower temperatures in the later nymphal stages would evidently be of considerable advantage to the species in permitting more of the larger nymphs, which occur abundantly late in the fall, to reach the adult stage, in which the insect passes the winter.

Some acceleration in rate of development of the beet leafhopper under alternating temperatures was observed in cases where the lower temperature was apparently well below the minimum for complete development (*9*). These differences in time cannot be explained on the basis of the foregoing discussion since the entire development must be assumed to have occurred at the higher temperature. Exclusive of diapause effects in exposure to cold, as reported by Bodine (*3*), Parker (*12*), Burdick (*6*), and others, evidence of a direct stimulus is shown by the results of exposure of other insects to temperatures below developmental zero, as in studies of Ludwig (*10*), Ludwig and Cable (*11*), and Cook (*7*). Cook found that the metabolism of cutworm larvae was accelerated at higher temperatures after exposure to cold near developmental zero and then gradually declined until a constant rate was reached. This effect was renewed in successive exposures to maintain an accelerated rate at the higher temperature.

The occurrence of successive growth phases that differ only in temperature coefficient may make it extremely difficult to determine the actual amount of stimulus or retardation produced under varying temperatures. As a result of this limitation in conventional methods of analysis based on duration of the stages, other time differences may be shown that are not attributable to a stimulus of temperature changes or, conversely, to an inhibitory influence of unnatural constant temperatures. Although problems of the relation of temperature to insect development are made more difficult, such effects of differences in temperature coefficient are important to an understanding of the influence of environment and to the proper interpretation of experimental results.

SUMMARY

The development of eggs and nymphs of the beet leafhopper (*Eutettix tenellus* (Bak.)) when exposed at one temperature for a given length of time and allowed to complete development at a second temperature was studied in the laboratory. The observed time was compared with that expected on the basis of the time required when the temperature was not changed.

A change from a higher to a lower temperature resulted in completion of development in a shorter time than was expected. This

effect was evidently not due entirely to a direct stimulus, since a reverse change between the same two temperatures usually resulted in an increase in the time required rather than a decrease. The differences in time required for development more logically indicated that the difference in growth rate at two temperatures was less in the earlier part of the embryonic and nymphal periods, and greater in the later parts, than was indicated by the average rates based on the time required for completion of the entire stages at each temperature.

Other studies of the time course of growth were made by weighing the nymphs daily during their development at different temperatures. When logarithms of the weights were plotted against time, the curves appear to consist of segments of different slope, which indicate that the growth rate differed in the five nymphal instars. The fact that the curves did not coincide when superimposed by expressing time and rate in percentages corroborated the previous results in showing the presence of two or more phases within the nymphal stage that are unequally affected by different temperatures.

LITERATURE CITED

(1) Alpatov, W. W.
1929. Growth and variation of the larvae of Drosophila melanogaster. Jour. Expt. Zool. 52: 407–432, illus.

(2) Bliss, C. I.
1926. Temperature characteristics for prepupal development in Drosophila melanogaster. Jour. Gen. Physiol. 9: 467–495, illus.

(3) Bodine, J. H.
1925. Effect of temperature on rate of embryonic development of certain orthoptera. Jour. Expt. Zool. 42: 91–109, illus.

(4) Brindley, T. A.
1930. The growth and development of Ephestia kuehniella Zeller (lepidoptera) and Tribolium confusum duval (coleoptera) under controlled conditions of temperature and relative humidity. Ent. Soc. Amer. Ann. 23: 741–757, illus.

(5) Brown, L. A.
1927. On the nature of the equation for growth processes. Jour. Gen. Physiol. 11: 37–42, illus.

(6) Burdick, H. C.
1937. The effects of exposure to low temperature on the developmental time of embryos of the grasshopper Melanoplus differentialis (orthoptera). Physiol. Zool. 10: 156–170, illus.

(7) Cook, W. C.
1927. Some effects of alternating temperatures on the growth and metabolism of cutworm larvae. Jour. Econ. Ent. 20: 769–782, illus.

(8) Crozier, W. J.
1926. On curves of growth, especially in relation to temperature. Jour. Gen. Physiol. 10: 53–73, illus.

(9) Harries, F. H.
1943. Some effects of alternating temperatures and exposure to cold on embryonic development of the beet leafhopper. Jour. Econ. Ent. 36: 505–509, illus.

(10) Ludwig, D.
1928. The effects of temperature on the development of an insect (popillia japonica newman). Physiol. Zool. 1: 358–389, illus.

(11) ——— and Cable, R. M.
1933. The effect of alternating temperatures on the pupal development of Drosophila melanogaster meigen. Physiol. Zool. 6: 493–508, illus.

(12) PARKER, J. R.
1930. SOME EFFECTS OF TEMPERATURE AND MOISTURE UPON MELANOPLUS MEXICANUS MEXICANUS SAUSSURE AND CAMNULA PELLUCIDA SCUDDER (ORTHOPTERA). Mont. Agr. Expt. Sta. Bul. 223, 132 pp., illus.
(13) PEAIRS, L. M.
1927. SOME PHASES OF THE RELATION OF TEMPERATURE TO THE DEVELOPMENT OF INSECTS. W. Va. Agr. Expt. Sta. Bul. 208, 62 pp., illus.
(14) POWSNER, L.
1935. THE EFFECTS OF TEMPERATURE ON THE DURATIONS OF THE DEVELOPMENTAL STAGES OF DROSOPHILA MELANOGASTER. Physiol. Zool. 8: 474–520, illus.
(15) TITSCHACK, E.
1925. UNTERSUCHUNGEN ÜBER DEN TEMPERATUREINFLUSS AUF DIE KLEIDERMOTTE (TINEOLA BISELLIELLA HUM.). Ztschr. f. Wiss. Zool. 124: [213]–251, illus.

U. S. GOVERNMENT PRINTING OFFICE: 1944